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Bacteriological and chemical quality of imported frozen chicken

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Abstract

A total number of forty samples of Brazilian imported frozen broiler, 20 of each trade mark were collected from main supermarkets in Cairo, Egypt and evaluated to determine the quality parameters examine each sample for deterioration criteria, then divide each into drip, skin and muscle then examine each separately microbiologically (*APC , total Staphylococcus , Staphylococcus aureus , Coliform*) then examined serologically for (*E.Coli, Salmonella*). The results showed that the drip % and T.B.A and T.V.B.N. are all within the permissible limits. The results obtained showed that the APC and coliform count of drip and skin and muscle of some samples both groups are higher than permissible limits which is may be due to bad storage and repeated thawing and freezing of samples, also founded *Staphylococcus aureus , E-Coli , Klebsiella , Proteus mirabilis, Proteus vulgaris ,* but failed to isolate *Salmonella.* The results revealed that group B is better than group A. The results showed that the skin has higher *APC* and *Staphylococcus aureus.* So for effective improvement of frozen broiler by hygienic slaughter, evisceration, washing, chilling and freezing, and by continuous stable freezing during storage at -18 °C.

Keywords: imported frozen broiler, deterioration criteria, microbiological quality.

Introduction:

Poultry meat is one of the most important sources of protein which ,characterized by relatively low production cost, easy digestibility, low fat content with high concentration of polyunsaturated fatty acids and high protein(**Chouliara *et al.*, 2007**). Therefore, poultry meat represents 30% and 38% of the world and Egyptian meat consumption, respectively. Freezing is one of the preferred methods of food preservation in the global meat export market, with a value of more than $13 billion per year (**Leygonie, et al., 2012**). Although freezing plays an important role in the extension of shelf life of meat, it has side effects on the quality of frozen meat. Frozen storage of chicken meat reduced their contents of moisture, total protein, total soluble protein (T.S.P), soluble protein nitrogen (S.P.N), soluble actomyosine nitrogen (S.A.N) and total lipids as the time of frozen storage progressed. It has been recorded that the thiobarbituric acid (TBA) values of chicken were found to increase during 6 months of frozen storage(**Shedeed *et al.*, 2006**). Microbial contamination of dressed chicken carcasses causes quality deterioration during storage and results in transmission of food borne pathogens that represent public health threats. *Salmonella spp. and Escherichia coli* are among the most important pathogenic bacteria that commonly contaminate raw poultry meat (**ICMSF, 2005 and Anang et al., 2009**). Nowadays, the intension of the meat industry in Egypt has been directed to the development of meat products from chicken

frozen chicken breast muscle ranged from 5.9 to 6.44 with mean value of 6.13±0.03 and the pH value of frozen chicken thigh muscle ranged from 5.9 to 6.55 with mean value of 6.21±0.04. A pH values ranged from 5.7 to 6.10 were recorded for breast and thigh chicken muscle during frozen storage at -18 °C for six months by **Shedeed (1999).** Slightly higher values, 5.8 and 6.6 were recorded for chicken breast and thigh, respectively by **Dainenk *et al.* (1989),** meanwhile, slightly lower values were recorded by **Ibrahim (2002)** who observed pH values of 5.91 and 5.77 for breast and thigh respectively and **Saad et *al.* (2013)** who recorded values of 5.77 for frozen chicken.

Thiobarbituric acid reactive substance (TBARs)

Lipid oxidation is easily occurred in chicken which is determined by analysis of thiobarbituric acid reactive substances. Lipid oxidation is one of the most important parameters that reflect deterioration degree and quality of raw and cooked meat products during refrigerated or frozen storage. The results recorded in Fig.(1) revealed that the TBARs of group A of examined imported frozen broiler has minimum value 0.17 and maximum value 0.36 with mean value 0.24 ±0.01, while group B recorded minimum value 0.15 and maximum value 0.46 with mean value 0.34±0.01. These results were in agreement with **Shedeed (1999)** who recorded that TBARs value of breast and thigh chicken muscle during frozen storage at -18 °C for six months ranged from 0.28 to 1.69 for breast chicken muscle while ranged from 0.36 to 2.47 mg/kg for thigh muscle and with **Oruc *et al.* (2005)** who obtained values of 0.25 mg/kg.. However, these results were higher than those of **Afifi (2000)** who recorded TBARs of 0.11 and 0.13 mg/kg for frozen chicken breast and thigh, respectively, **Conchillo *et al.* (2005)** who obtained values of 0.01-0.03 mg/kg for chicken breasts stored at -18°C for 3 months under aerobic and vacuum condition, **Saad *et al.* (2013)** who observed values of value 0.09±0.01 mg /kg and **Ibrahim (2002)** who recorded TBARs of 0.04±0.01 mg /kg. Higher values for TBARs of 0.31 and 0.51 mg/kg were recorded for frozen chicken breast and thigh by **Moawad (1987).** The obtained TBARs values were nearly within the permissible limits of Egyptian organization for standardization and quality control (E.O.S.Q.C. 1090-2005):- thiobarbituric acid not exceeds 0.9 mg/kg of chicken meat.

Total volatile base nitrogen (TVBN)

The results in table and figure (1) showed that the TVBN of group A of examined imported frozen broiler ranged from 12.6 to 17.64 with mean value of 15.07±0.309. Moreover, the TVBN of group B ranged from 8.96 to 16.8 with mean value of 13.67 ±0.504 mg /100gm. Slightly lower values were obtained by **Afifi (2000)** who recorded TVBN values of 13.87 and 12.57 for frozen chicken breast and thigh, respectively, **Ibrahim (2002) and Saad *et al.* (2013)** who recorded TVBN of 11.29±0.32 and 9.11±0.33 mg/100g, respectively. The obtained results in this study were nearly within the permissible limits of Egyptian organization for standardization and quality control (E.O.S.Q.C. 1090-2005) which stated that the TVBN should be not more than 20 mg /100gm.

Taple (1) : deterioration criteria of examined two group of imported frozen broiler (N=20):

Examined imported frozen chicken	pH value		T.B.A	T.V.B.N
	Breast	Thigh		
Group A	5.76±0.01	6.46±0.01	0.24±0.01	15.10±0.31
Group B	5.76±0.01	6.49±0.01	0.34±0.16	13.68±0.50

raw chicken. The research on the quality of imported frozen

chicken still limited, therefore, the main objectives of the current study were to study the microbiological quality and deterioration criteria of imported frozen chicken in the Egyptian supermarkets.

Materials and Methods:

Sample collection

A total of forty samples of whole frozen chicken imported from Brazil were collected from different supermarkets in Cairo. The samples represented two different types of frozen chicken 20 from (group A) and 20 from (group B). Samples were transferred in cooling ice box to the laboratory of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Cairo University for examination. The collected samples were thawed completely in refrigerator at 4 °C. After thawing, skin and muscles (breast and thigh) were separated for microbiological and chemical analysis.

Deterioration criteria

The pH value measured by homogenizing five grams from each muscle sample (breast and thigh) with 20 ml distilled water for 10-15 seconds and measured using pH meter (Lovibond Senso Direct) with a probe type electrode (Senso Direct Type 330) **(Kandeepan et al., 2009).** Thiobarbituric Acid Reactive Substances (TBARs) were measured using five grams mixed muscle samples of breast and thigh which were homogenized with 15 ml deionized distilled water using a stomacher (Lab blender 400) according to the method of **Du and Ahn (2002).** Total Volatile Basic Nitrogen (TVBN) was measured using the macro-Kjeldahl distillation method according to **Kearsley et al. (1983).**

Microbiological examination

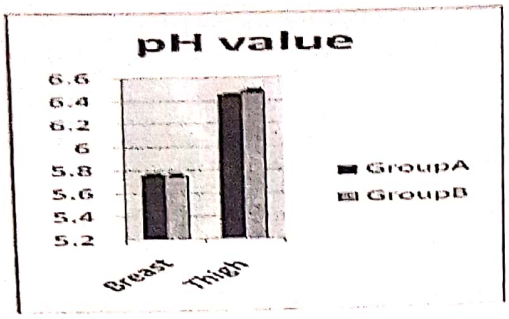
Samples prepared by mixing 25 gm of sample + 225 ml of ringer solution. The prepared samples were analyzed for the following microbiological criteria :Total aerobic plate count were counted and calculated by **(APHA, 1992)**by inoculate loopful on standard plate count agar plates. Staphylococcus aureus were counted according to **(FAO, 1992)** by inoculation on standard Baird Parker agar. Salmonella were examined according to **(ISO 6579, 2002)** by pre enrichment on buffer peptone broth then enriched on Rappaports Vassiliadis broth then plating on Xylose-Lysine Desoxycholate (XLD) agar, then the positive colonies were exposed to further biochemical and serological examination. Coliform are counted according to **(ISO 4832:2006)** by inoculation on Violet Red Bile Lactose agar VRBL Agar, then the positive colonies were exposed to further biochemical and serological examination.

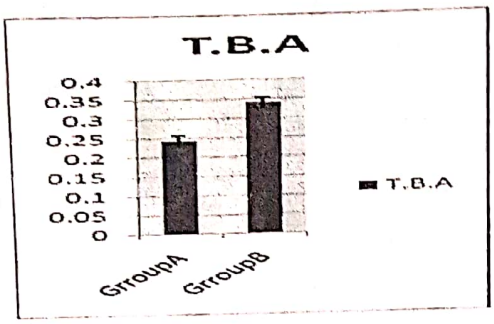
Results and Discussion

Deterioration criteria:

pH value

The results recorded in Fig. (1) revealed that the pH values of breast muscle of examined imported frozen broiler group A had minimum value of 5.7 and maximum value of 5.84 with mean value of 5.76 ±0.01. Meanwhile, the pH value of thigh muscle of the same group was with minimum value of 6.38 and maximum 6.57 with mean value of 6.46±0.01. The pH values of breast muscle of group B recorded minimum value of 5.58 and maximum value of 5.83 with mean value of 5.75 ±0.01 & the pH value of thigh muscle of the same groups showed minimum value of 6.38 and maximum 6.59 with mean value of 6.48±0.01. The obtained results were in agreement with **Afifi (2000)** who recorded a pH value of





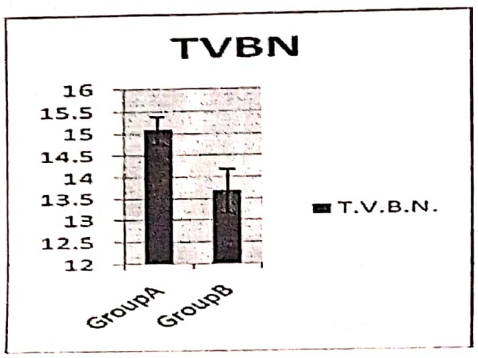


Fig. 1 Deterioration criteria of imported frozen chicken

Microbiological examination:

Aerobic plate count (APC)

The results recorded in table and figure (2) showed that the APC of examined drip of imported frozen broiler of group A ranged from 3.3 to 5.63 with mean value 4.51±0.15 log cfu/mL. Moreover, those of group B ranged from 3.0 to 5.89 and mean value 4.09±0.20. The APC of examined skin ranged from 2.77 to 5.0 with mean value 4.04 ±0.14 log cfu/g for group A. However, APC of examined skin of group B ranged from 3.0 to 5.79 with mean value 4.07 ±0.19 log cfu/g. Moreover, the APC of muscle(homogenized mix of breast and thigh muscle) of group A ranged from 3 to 5 with mean value 3.69±0.15 log cfu/g and those of group B ranged from 2 to 5.7 with mean value 3.62 ±0.25 log cfu/g. The obtained results were in agreement with **kozacinski *et al.* (2006)** who recorded that the APC in frozen ground chicken meat was 5.23±0.50 log cfu/g and **Hassan (2015)** who recorded APC value of 5.53 log cfu/g for frozen chicken carcasses. However, these results were higher than those of **Zaki (1994)** who reported that the APC (log cfu/g) were 2.47 before washing, 2.49 after chilling and 1.7 after freezing, **Shareef *et al.* (2012)** who obtained APC of 3.81±0.25 log 10 cfu/cm^2 for imported chicken thigh, **Altalhi *et al.* (2004)** who obtained APC 3.47 log cfu/cm^2 for frozen chicken meat and **Willayat *et al.* (2006)** who obtained APC 1.19 log cfu/g for frozen chicken. However, the obtained results were lower than those recorded by **Mansour (1995)** who reported aerobic plate count of 6.55 log cfu/g. The presence of higher counts of aerobic bacteria indicates higher proportion of contamination, poor hygiene procedures during slaughter and handling as well as possible interruptions in the deep freezing process.

Table (2) APC (log cfu/g) of Drip, Skin and Muscle of imported frozen broiler (N=20):

Examined imported frozen chicken	Drip	Skin	Muscle
GroupA	4.51±0.13	4.04±0.14	3.69±0.15
GroupB	4.09±0.20	4.07±0.19	3.62±0.25

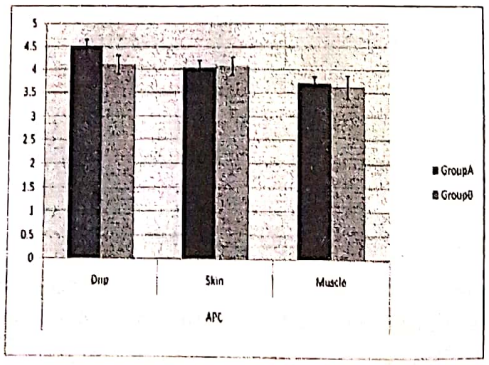


Fig. (2): Aerobic plate counts of imported frozen chicken

***Staphylococcus aureus* counts**

The obtained results in table and fig (3) showed that the total *Staphylococcus aureus* counts for the drip of examined imported frozen broiler of group A ranged from 2.69 to 4.22 with mean value 3.43±0.12 log cfu/ml but those of group B ranged from 2.0 to 3.95 with mean value 2.96±0.16 log cfu //mL. The total *Staphylococcus aureus* (log cfu/g) for the skin of examined imported frozen broiler of group A ranged from 2.0 to 4.30 with mean value 2.88±0.13 and the total *Staph. aureus* (log cfu/g) of skin of group B ranged from 2.3 to 3.72 with mean value 3.11 ±0.13. Moreover, the total *Staph. aureus* (log cfu/g) for the muscle of examined imported frozen broiler of group A ranged from 2.0 to 3.30 with mean value 2.38±0.151 and those of group B ranged from 2.0 to 3.39 with mean value 2.54 ±0.12. The results revealed that 3 (15%) and 5 (25 %) of the isolated *Staphylococcus aureus* group A and B; respectively were positive for coagulase teat. The obtained results in this study were in agreement with **Mansour (1995)** who reported total *Staph.aureus* 3.04±2.61 for frozen chicken, **Kozacinski *et al.* (2006)** who reported total count of *S. aureus* of frozen ground chicken meat ranged from 1.70 to 3.69 cfu/g. The obtained results were higher than those of **Zaki (1994)** who observed that the total *Staph. aureus* counts of examined frozen broiler skin & muscle were 1.98 &1.50, respectively and **Shareef *et al.* (2012)** who recorded *Staphylococcus aureus* count of 0.63±0.05 log cfu/cm^2 for imported frozen chicken thigh. *Staph. aureus* were isolated in 16.66% from imported thighs. However, higher counts for *Staph. aureus* were recorded by **Hassan (2015)** who obtained total *Staphylococcus aureus* count of 4.11 log cfu/g for frozen chicken carcasses. Different percentages for isolation of coagulase positive *Staph. aureus* were recorded by different authors. In this respect, **Mohamed *et al.* (2010)** isolated *Staph. aureus* from 80 sample of fresh poultry meat in percentage of 47.5% while the coagulase positive *staph. aureus* was detected in 27.5 % of tested samples. The obtained results revealed that the muscle had the least count of *staphylococcus* which, indicated that the microbial contamination was from processing tool, equipments, human contact and carcass to carcass contact.

Table (3): Total *Staph.* (log cfu/g) of Drip, Skin and Muscle of imported frozen broiler (N=20):

Examined imported frozen chicken	Drip	Skin	Muscle
GroupA	3.43±0.12	2.88±0.14	2.38±0.15
GroupB	2.96±0.16	3.10±0.13	2.53±0.17

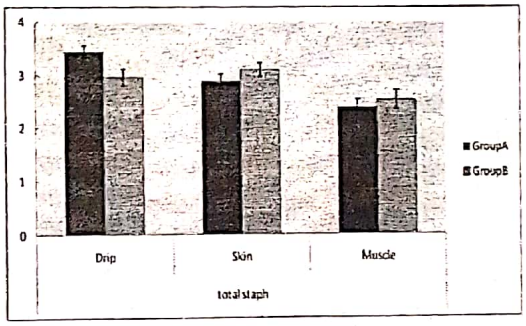


Fig. (3): Total *Staphylococcus* counts of imported frozen chicken

Salmonella

Salmonella failed to be isolated from any of the examined samples of group A and group B. These results were in agreement with **EL Nawawy *et al.* (2012)** who failed to isolate *Salmonella* from imported & locally frozen chickens. However, On the other hand, many previous authors isolated *Salmonella* in different percentages from frozen poultry including **Norberg (1981)** who isolated *Salmonella* from 1.2% of frozen chicken sample, **kozacinski *et al.* (2006)** who isolated *Salmonellae* from 10.53% of samples of

frozen ground chicken. **Willayat *et al.* (2006)** who recovered *Salmonella spp* from 4% of samples of frozen chicken. **Tessari *et al.* (2008)** who recorded incidence 0.81 % *Salmonella enteritidis* from frozen chicken and **Moussa *et al.* (2010)** who isolated *Salmonella* from locally frozen chicken and imported frozen chicken with incidence of 7.89% and 4.83%, respectively. The incidence of *Salmonella* in chicken meat in different countries was reported as 8.05 % in Egypt, 13.4% in France, 9.7% in Denmark, 6.8 % in USA, 5% in west Germany and 3.43% in Brazil (**Safwat *et al.* 1985**)

Coliform and *Escherichia coli*

The results observed in table and fig. (4) showed that the coliform counts (log cfu/g) for the drip of examined imported frozen broiler of group A ranged from 2 to 3.6 with mean value 2.56±0.17 and those of group B ranged from 2.0 to 3.7 with mean value 2.48±0.31. However, the coliform counts for the skin of examined imported frozen broiler of group A ranged from 2.0 to 3 with mean value 2.32±0.11 and those of skin of group B ranged from 2 to 3.3 with mean value 2.46±0.204. Moreover, the coliform counts for the muscle of examined imported frozen broiler of group A ranged from2.6 to 2.8 with mean value 2.72±0.12 and those of muscle of group B were less than 2. Serological examination revealed only 2 cases of *E. coli* (*E. coli* poly (6) O: 169 and *E. coli* –ve poly 1 to poly 8. These two cases were isolated from group A with incidence rate of 5% of *E. coli* from imported frozen chicken. *E. coli* was isolated previously by many authors in different rates. In this respect, **Mansour (1995)** reported coliform count of 3.22 log cfu/g from frozen chicken with incidence of isolated *E. coli* strains was 6.58% and the isolated strains were considered as untypable. Incidence rate of 8.8% was isolated from frozen chicken by **Tolba (2000). EL Nawawy *et al.* (2012)** revealed that the coliform count (MPN) of examined frozen imported chicken was 2 log/ g and that of locally frozen chicken was 1.8 log cfu/g and the incidence of *E. coli* was 12%, 10% respectively. **Hassan (2015)** recorded total *coliform* count in frozen chicken carcasses of 2.15 log cfu/g, furthermore, pathogenic *E. coli* could be isolated from frozen chicken carcasses samples with an incidence of 72% and the identified strains of *Enteropathogenic E. coli* from examined frozen chicken carcasses were O26:K60, O86:K61, O119:K69, O126:K71 and O124:K72. Lower incidence rates were recorded by **Zaki (1994)** who obtained coliform count of 1.31 log cfu/g and *E-coli* couldn't be isolated from all of the examined samples of skin and muscle. However, *E. coli* O157: H 7 failed to be isolated from frozen chicken **by Ingham *et al.* (2005) and Hassouba *et al.* (2007).** The obtained data could be concluded that the coliform *and E. coli* species in frozen chicken may be indicative of defective technique applied during preparation, handling, processing and storage lead to contamination with spoilage and pathogenic microorganisms of faecal origin.

Table (4) coliform (log cfu/g) of Drip, Skin and Muscle of imported frozen broiler (N=20).

Examined imported frozen chicken	Drip	Skin	Muscle
GroupA	2.56±0.17	2.32±0.11	<2±0.00
GroupB	2.48±0.31	2.47±0.20	2.72±0.12

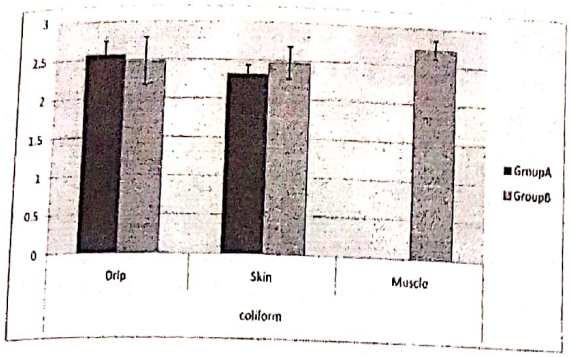


Fig. (4): Coliforms of imported frozen chicken.

Proteus and Klebsiella

The date recorded in table(5) showed that *Proteus mirabilis* was isolatedfrom5 samples of group A of examined frozen imported broiler (12.5 %), *Proteus vulgaris*was isolated from 1 sample (2.5 %) and *Proteus mirabilis* was isolated from 5 samples of group B (12.5 %). *Proteus mirabilis* was isolated **by EL Nawawy *et al.* (2012)**from 10% of imported frozen chicken and 2 % of locally frozen chicken. *Klebsiella* was isolated from only one sample of group A (2.5 %), however, **EL Nawawy *et al.* (2012)** previously isolated *Klebsiella* from 4% of locally frozen chicken.

Table (5): *Proteus and Klebsiella* of Drip, Skin and Muscle of imported frozen broiler (N=20).

Examined Imported frozen chicken	Microorganism	No.	%
GroupA	Klebsiella	1	0.5%
	Proteus mirabilis	5	25%
	Proteus vulgaris	1	0.5%
Group (B)	Proteus mirabilis	5	25%

Conclusion:

From the obtained data it could be concluded that the presence of *aerobic* bacteria indicates higher proportion of contamination, poor hygiene procedures during slaughter and possible interruptions in the deep freezing process. The *coliform and E.Coli* species in frozen chicken may be indicative of defective technique applied during preparation, handling, processing and storage lead to contamination with spoilage and pathogenic microrganisms of faecal origin. It could be also concluded that the microbial contamination lead to economic losses.

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الملخص العربى

اجريت هذه الرساله على ٤٠ عينة دجاج بواقع ٢٠ عينه من المجموعه أ و٢٠ عينه من المجموعه ب واجريت عليهم الفحص الظاهرى وكانوا جميعا بحاله ظاهريه جيده ثم اختبار تحديد نسبة السائل المنفصل بعد الاذابه من التجميد بمتوسط 2,82% ±0,11% للمجموعه أ و 3,34%±0,22% للمجموعه ب وكانوا فى الحدود المسموح بها وفقا للمواصفه القياسيه المصريه ١٠٩٠ لسنه ٢٠٠٥ ثم اختبار قيمة تركيزايون الهيدروجين الموجب وكانت بمتوسط 0.00896±5,762 لصدور المجموعه أ و 0,0109±6,456 للفخد بينما تركيز ايون الهيدروجين الموجب لصدر المجموعه ب 0,0145±5,757 و للفخد 0,0738±6,487 .خضعت هذه العينات لاختبارات درجة فساد العينه , حمض الثايوباربتيوريك وكان بمتوسط 0,0139±0,242 للمجموعه أ بينما كان بمتوسط 0,0165±0,340 كما تم اجراء اختبار تحديد قيمة القواعد النيتروجينيه الكليه للطيارة وكان بمتوسط 0,309±15,07 للمجموعه أ بينما كانت 13,67 ±0,504 للمجموعه ب .

تم تقسيم كل عينه الى سائل منفصل وجلد وعضلات واجريت الاختبارات الميكروبيولوجيه على كل منهم على حده والنتائج اوضحت ان عد البكتيريا الهوائيه كان للسائل المنفصل للمجموعه أ بمتوسط 4,512 ±0,152 و بمتوسط 0,204±4,091 للمجموعه ب بينما كان عد البكتريا الهوائيه للجلد للمجموعه أ بمتوسط 0,14±4,04 و بمتوسط 4,067 ±0,189 للمجموعه ب ولكن كان عد البكتيريا الهوائيه للعضلات بمتوسط 0,148±3,694 للمجموعه أ و 0,250±3,617 للمجموعه ب .النتائج اوضحت ان العد الكلى للاستافيلو كوكس للسائل المنفصل للمجموعه أ كان بمتوسط 0,120±3,431 و2,962 ±0,159 للسائل المنفصل للمجموعه ب بينما كان متوسط العد الكلى للاستافيلو كوكس للجلد للمجموعه أ 0,135±2,88 و 0,129±3,102 للمجموعه ب كما كان متوسط العد الكلى للاستافيلو كوكس للعضلات للمجموعه أ 0,151±2,38 بالمقارنه ب 0.117± 2,536 للمجموعه ب .النتائج المعطاه اوضحت ان الاستافيلوكوكس اوريس (ميكروب التسمم الغذائى)كان بنسبة 15% للمجموعه أ مقارنه ب 25% للمجموعه ب. اجريت الاختبارات السيرولوجيه على العينات لفحص ميكروب السالمونيلا وكانت العينات كلها سلبيه للسالمونيلا.

النتائج المسجله اوضحت ان متوسط عد الميكروب القولونى للسائل المنفصل للمجموعه ا 0,186±2,564 و 2,479 ±0,309 للمجموعه ب كما كان عد الميكروب القولونى للجلد للمجموعه أ بمتوسط 0,107±2.317 مقارنة ب 2,46 ±0,204 للمجموعه ب وكان عد الميكروب القولونى للعضلات بمتوسط 0,120±2,72 مقارنه ب اقل من 2 للمجموعه ب .الفحص السيرولوجى الذى اجرى على العينات اوضح ان هناك حالتين فقط للايشريشيا كولاى وكانوا فى المجموعه أ وذلك بمعدل نسبة حدوث 1% كما اوضحت ووجود ميكروب البروتيس ميرابيليس للمجموعه أ بنسبة 25% وكذلك للمجموعه ب بينما وجدت البروتيس فالجاريس فى المجموعه أ فقط بنسبة 0,5 % وكذلك الكليبسيلا فى المجموعه أ فقط بنسبة 0,5% .

لذلك يجب التنبيه على الشركات بضرورة اتخاذ الاجراءات الصحيه المحكمه والتعقيم اللازم اثناء الذبح والتنظيف والتعبئه كما يجب التنبيه باهيمة النقل والتخزين فى ثلاجات التجميد على درجة حرارة -18 وان تكون بصفه مستمرة , ويجب التخزين فى الثلاجات فى درجة حرارة ثابته -18 وتفادى الاذابه واعادة التجميد .