EFFECT OF CHILLING ON SOME BACTERIOLOGICAL CHANGES IN FRESH BEEF SAUSAGE

A. M. Ahmed*; Kh. I. Sallam ; M. A. M. Yassien***

**Department of Food Hygiene & Control, Faculty of Vet. Med., Suez Canal University.*

***Department of Food Hygiene & Control, Faculty of Vet. Med., Mansoura University., Egypt.*

ABSTRACT

30 fresh locally manufactured beef sausage samples were randomly collected from different butcher's markets from Ismailia city and stored at chilling temperature (4°C) then evaluated for post-production bacteriological changes. The mean log values for total psychrotrophic counts were 3.68, 3.95, 5.23, 6.11 and 5.28 cfu/g, while for total lactic acid bacterial counts they were 2.36, 2.58, 4.64, 5.57 and 7.64 cfu/g and for total enterobacteriaceae count were 2.56, 3.22, 3.73, 3.95 and 2.17 cfu/g at 0, 2, 4, 6 and 8 days post storage, respectively. The mean values of total psychrotrophic counts for beef sausage samples were significantly different (P < 0.05) allover the storage periods while the total lactic acid bacterial counts were not significantly increased up to 2 days of chilling storage then their counts were significantly increased (P < 0.05) to reach maximum value (log 7.64 cfu/g) after 8 days of storage at chilled temperature (4°C). On the other hand, the total enterobacteriaceae count in the examined sausage samples significantly decreased (P < 0.05) to log mean value 2.18 cfu/g at the end of storage period (8 days). Sausage productions by local butcher's markets required a considerable action to increase its quality and shelf-life. Some suggestions and recommendations for improving sausages qualities were discussed.

INTRODUCTION

Fresh beef sausage is one of the most palatable meat products, which mainly composed of fresh beef usually uncured, comminuted, seasoned, stuffed into casings and must be cooked fully before serving **(Stamer, 1976)**. In Egypt, it plays an important role in economic terms due to its considerable price and high market demands.

The shelf-life of fresh sausage is limited due to the absence of anti-microbial substances such as nitrite. In addition, temperature is a key for bacterial growth. Psychrotrophic bacteria, including lactic acid bacteria and some enterobacteriaceae group, grow logarithmically at 4°C, which

means that chilled sausage have a drastically shortened shelf-life the longer it remains in temperatures at 4°C (**Kraft, 1992**). Microbial growth, especially psychrotrophs, in the fresh sausage together with activity of the meat endogenous enzymes are undoubtedly partially responsible for the development of a number of aromatic and sapid compounds (**Ordonez,et al. 1999**). Moreover, these microbial growthes, when increased under favorable conditions they particularly responsible for development of product's spoilage. In addition, such microorganisms may produce heat resistant enzymes that may continue to cause deterioration during later storage even if the bacteria do not more survive (**Gilliland et al., 1984; Borch et al., 1996 a.d Hierro et al., 1999**).

Leroy (2002) reported that although predictive microbiology generally focuses on the potential outgrowth of spoilage bacteria in foods, little attention has been paid to lactic acid bacteria. Lactic acid bacteria are a group of related bacteria that produce lactic acid as a result of carbohydrate fermentation. Also they have the ability to grow under a variety of environmental conditions which allow them to be highly competitive in meat products (**Stamer, 1976**).

Family Enterobacteriaceae has been suggested as indicator of faecal contamination of meat products with the idea that such contamination might possibly be detected even coliforms are not present or viable (**Hayes, 1992**). Fresh sausage usually has a high microbial population could be ranged from 10^4 to $5x10^8/g^{-1}$ (**Kraft, 1992**). **Khalafalla and El-Sherif (1993)** reported that the mean counts of psychrotrophic bacteria, enterobacteriaceae and lactic acid bacteria in randomly collected sausage samples from retail markets were 2×10^5, 6×10^3 and 8×10^2 organisms per gram, respectively.

The purpose of this study was to evaluate the changes in total psychrotraphic, lactic acid bacterial and enterobacteriaceae counts post-production of the local manufacture fresh beef sausages and during storage at chilled temperature (4°C).

MATERIALS AND METHODS

1. Samples :

30 fresh beef sausage samples were randomly collected from the butcher's markets in Ismallia City, directly post-production. Each sample, nearly weighted 200g, was individually wrapped in clean sterile polyethylene bag then transferred immediately in ice-box to Food Hygiene Laboratory, Suez Canal University. The samples were stored in chiller at 4°C. A part from each sample was subjected to microbiological evaluation in periodical interval at 0, 2, 4, 6 and 8 days post storage.

2. Bacteriological analysis (APHA, 1992)

Ten grams from the core of each sausage sample were aseptically sampled, diluted with 90 ml of sterile 0.1% peptone water (w/v) in a sterile stomacher bag and homogenized in a Stomacher (LAB-BLENDER, 400, London, UK) for one minute to provide dilution of 10^{-1}. From the original homogenate, 1 ml was transferred asceptically to a test tube containing 9 ml sterile 0.1% peptone water (w/v) to prepare dilution of 10^{-2}, then from which further ten fold decimal serial dilution up to 10^{-8} were prepared. From these serial dilutions, the bacteriological investigations were performed.

2.1. Total Psychrotrophic Count

The pouring technique recommended by **APHA (1992)** was used. One ml from each dilution was inoculated into duplicate sterile Petri dishes then about 15 ml of 45°C standard plate count agar were poured, left to solidify and incubated at 7°C for 10 days. The average count of duplicate plates was taken and the total psychrotrophic count was expressed as cfu/g.

2.2. Total Lactic acid Bacterial Count

The pouring technique recommended by APHA (1992) was used. One ml from each dilution was inoculated into duplicate sterile Petri dishes then about 15 ml of 45°C de Man Rogosa Sharp (MRS) agar were poured, left to solidify and incubated at 32°C for 48h in anaerobic jar. The average count of duplicate plates was taken and total lactic acid bacterial count was expressed as cfu/g.

2.3. Total Enterobacteriaceae Count

The technique was recommended by **ICMSF (1978)**. From each of the previously prepared serial dilution, 0.1 ml aliquots were delivered into duplicate sets of Petri dishes, previously inoculated by 15 ml of sterile violet red bile glucose agar. After sufficient spreading, a cover layer of 10 ml of the medium was poured over all the plates. The plates were incubated at 37°C for 24 hour. The expected colonies "clear visible purple colony surrounded by a halo purple zone" were counted and the results expressed as cfu/g.

3. Identification of Isolates

Five representative colonies were randomly picked from each plate of psychrotrophic, lactic

acid bacteria and enterobacteriaceae group. Each colony was isolated for further purification and identification by using biochemical tests according to **MacFadyean (1988)**

4. Statistical Analysis

The obtained data were subjected to a one way analysis of variance (SPSS 6.1.2. 1995) to study the significance among the mean values of total psychrotrophic, lactic acid bacteria and enterobacteriaceae counts in chilled sausage samples under the different stages of bacteriological analysis.

RESULTS AND DISCUSSION

In the recent years, sausages manufacturing is based on a highly sophisticated science depending on the high quality of the ingredients used in the processing to safe the consumers from foodborne pathogens as well as to prolong the shelflife of the final products **(Price and Schweigert, 1987)**. The obtained results in this study revealed that fresh beef sausage constitute a considerable initial (0 day). psychrotrophic, lactic acid bacteria and enterobacteriaceae counts. This clearly indicated that the production of fresh sausages by local butchers in Ismailia city is done under inadequate sanitary and hygienic measures.

Differential bacterial counts :

The mean log values for total psychrotrophic, total lactic acid bacteria and total enterobacteriaceae counts in fresh beef sausage stored at chilling temperature of 4°C were shown in Table (1). It is evident that the mean log values for total psychrotrophic count were 3.68, 3.95, 5.23, 6.11 and 5.28 cfu/g at 0, 2, 4, 6 and 8 days post processing respectively. The mean log values for total lactic acid bacterial counts were 2.36, 2.58, 4.64, 5.57 and 7.64 cfu/g at 0, 2, 4, 6 and 8 days post processing respectively, whereas the respective mean log values for total enterobacteriaceae counts were 2.56, 3.22, 3.73, 3.95 and 2.17 cfu/g at 0, 2, 4, 6 and 8 days respectively. **Khalafalla and El-Sherif (1993)** recorded nearly similar mean count values in randomly collected beef sausage samples from retail food markets. Chilling storage did not prevent but delay the microbial growth in fresh sausage and usually higher microbial content was detected in sausage after prolonged storage at low temperatures **(Korkeala et al., 1989)**. Psychrotrophic and other spoilage microorganisms when reach considerable high counts they cause defects in meat products such as sour, off-flavour, discolouration, gas production, slime production and decrease in pH **(Borch et al., 1996)**.

Figure (1) declared the changes in total psychrotrophic, lactic acid bacterial and enterobacteriaceae counts in fresh sausage during storage at chilling temperature (4°C). The mean values of total psychrotrophic counts for beef sausage samples were significantly different ($P < 0.05$) allover the storage periods. The psychrotrophic bacteria continue to grow in the beef sausage till reach highest counts (log 6.11 cfu/g) after 6 days storage under chilling temperature (4°C), then the counts significantly ($P<0.05$) decreased (log 5.28 cfu/g) at the end of storage period (8 days).

Frequency distribution of existed psychrotrophic bacteria :

The frequency distribution of most identified psychrotrophic bacteria isolated from examined sausage samples were listed in table (2). The most identified psychrotrophic spp. from sausage samples at 0 day were Acinetobacter spp. 3 (3.6%), Aeromonas spp. 8 (9.6%), Bacillus spp. 15 (18.1%), Coliform 4 (4.8%), Enterobacter spp. 4 (4.8%), Lactobacillus spp. 2 (2.4%), Leuconostoc spp. 3 (3.6%), Micrococcus spp. 12 (14.5%), Pseudomonas spp. 5 (6%), Staphylococcus spp. 21 (25.3%) and Streptococcus spp. 6 (7.3%). Acinetobacter spp., Aeromonas spp., Coliform and Enterobacter spp. were not detected after 4 days from chilling storage at 4°C. The predominated psychrotrophic spp. in sausage samples after 8 days chilling storage were Lactobacillus spp. 45 (42.1%) and Leuconostoc spp. 30 (28%).

Reduction in temperatures is one of the most important means of restricting bacterial growth and is of particular concern in considering psychrotrophic spoilage organisms and pathogens (**Kraft, 1992**). The decrease in psychrotrophic counts in the examined sausage samples could be resulted from the effect of some additives like sodium chloride and the changes in the pH of the products on the psychrotrophic growth (**Gilliland and Speck, 1975**). The production of acid by lactic acid bacteria is one of the first manifestations of their growth in sausage. Lactic acid is the one produced at higher concentrations, although other acids are produced in minor quantities, among them is the lactic acid which probably the most important. Acetic acid, while being present in small concentrations, is particularly toxic to some bacteria (**Pinheiro et al., 1968**).

Regarding to the total lactic acid bacterial counts in sausage samples stored at chilling temperature (4°C), figure (1) revealed the log mean values of total lactic acid bacterial counts were not significantly increased up to 2 days chilling storage then the counts significantly increased ($P < 0.05$) to reach a maximum value of log 7.64 cfu/g after 8 days at chilling temperature (4°C).

Frequency distribution of isolated lactic acid bacteria:

Lactic acid bacteria as presently constituted consist of 4 genera; lactobacillus, Leuconostoc, Pediococcus and Streptococcus. (**Jay, 1986**). These organisms are wide spread in nature and

the natural habitat of some is unclear.

The frequency distribution of most identified lactic acid bacteria isolated from sausage samples at 0 day, were Lactobacillus spp. 10 (34.5%), Leuconostoc spp. 8 (27.6%), Pediococcus spp. (20.7%) and Streptococcus spp. 5 (17.2%), while the recorded frequency for such isolates after 8 days chilling storage at 4°C were reached to 55 (34.8%), 46 (29.1%), 42 (26.6%) and 15 (9.5%) respectively (Table 3).

It is noticed that lactic acid bacteria were the predominant microorganisms during refrigerated storage and that is expected in sausage products (**Nychas and Arkoudelos, 1990**). The contamination of sausage by lactic acid bacteria occurred as a result of the manufacturing and handling processes, the environment and working surfaces contributed to sausage contamination of various types of lactic acid bacteria (**Dykes, et al.1991**).

Samelis and Georgiadou (2000) indicated that Lactobacillus and Leuconostocs spp. were occurred at considerable higher incidences (92-96% and 14-21% respectively) in chilled sausage stored at 4°C whereas Pseudomonas and Micrococcaceae spp. grew, but failed to increase above 10^5 cfu/g in all samples during chilling storage. Lactic acid bacteria are responsible for decrease of pH of the sausages during chilling storage (**Lucke, 1989**). Regardless the use of lactic acid bacteria as a major potential biopreservation for some foods of animal origin because they are safe to consumer, they naturally dominate the microflora of many foods during its storage (**Stiles, 1996**), they have been implicated in outbreaks of foodborne illness and share public health hazard (**Franz et al., 1999**).

On the other hand, the enterobacteriaceae counts in the examined sausage samples were significantly increased ($P < 0.05$) under chilling storage up to maximum log mean values (3.73 and 3.95 cfu/g at 4 and 6 days respectively), then decreased significantly ($P < 0.05$) to log mean value 2.18 cfu/g, after 8 days, at the end of storage period (figure 2).

Frequency distribution of identified enterobacteriaceae :

The mostly identified enterobacteriaceae isolated from sausage samples at 0 day storage were Citrobacter freundii, 16(16.7%); Enterobacter spp., 22(22.9%); Escherichia coli, 13 (13.5%); Hafnia alvei, 4 (4.2%); Klebsiella spp., 8 (8.3%); Proteus spp., 17 (17.7%); and Serratia spp., 16 (16.7%). It is worthy to mention that the detection of coliforms (E. coli, Enterobacter, Citrobacter and Klebsiella) in the sausage samples at 0 day storage is indicators of fecal contamination of the products during processing (**Kraft, 1992**). Fortunately, the growth of the family enterobacteriaceae may be suppressed by salt or presence of lactic acid bacteria at refrigerated temperature

(Gill, 1986). After 4 days chilling storage of sausage at 4°C, Hafnia alvei, Klebsiella spp. and Serratia spp., were not recorded, while other isolates appeared to decrease in their frequency distribution. After 8 days chilling storage of sausage samples, the frequency distribution was Citrobacter freundii 6 (27.3%) Enterobacter spp. 5 (22.7%), Escherichia coli, 2 (9.1%), and Proteus spp. 9 (40.9%). The lowering of pH by the lactic acid bacteria produces a less favorable environment for growth of undesirable bacterial types, particularly those that are psychrotrophic and putrefactive types as Pseudomonas spp. and pathogens as Staphylococcus spp. **(Speck, 1979)**. In addition, some lactic acid bacteria as lactobacilli seem to produce large inhibitory amount of H_2O_2 **(Price and Lee, 1970)**. The H_2O_2 produced is inhibitory to a number of Gram negative and Gram positive species **(Speck, 1979)**.

From this study, it can be concluded that fresh sausages collected from Ismailia city were exposed to more extensive handling and processing during manufacture, which appeared to have high microbiological counts. Good manufacturing practices and sanitation standard operating procedures are keys for reducing microbiological contamination in meat products. Most retail stores do not have functional quality assurance programs, as evident in the higher microbiological contamination in final products. It is likely that development of retail Hazard Analysis Critical Control Point plans would be beneficial to ensure the safety of fresh sausage. Furthermore, butcher shops operations should take a closer look at how completely they are cleaning and sanitizing grinding equipment. Further studies are warranted to identify efficient means for removing bacterial contamination from equipment in both processing plants and retail stores.

Table (1) : Log mean values of total psychrotrophic, lactic acid bacteria and enterobacteriaceae counts (±S.E.) for beef sausage stored at chilling temperature (4°c)

	Total Psychrotrophic counts			Total Lactic acid bacteria counts			Total Enterobacteriaceae counts		
Time	Mean (± S.E.)	Mini.	Max.	Mean (± S.E.)	Mini.	Max.	Mean (± S.E.)	Mini.	Max.
0 day	3.68 ±2.62	2.43	5.21	2.36 ±0.86	0.83	3.61	2.56 ±2.08	0.88	4.72
2 days	3.95 ±2.85	3.21	6.24	2.58 ±2.11	2.11	5.28	3.22 ±2.64	2.61	5.34
4 days	5.23 ±4.23	3.99	7.31	4.64 ±2.97	3.37	6.84	3.73 ±3.24	3.74	6.21
6 days	6.11 ±3.61	4.29	7.28	5.57 ±3.56	4.76	7.04	3.95 ±2.34	2.11	4.37
8 days	5.28 ±3.34	4.33	7.33	7.64 ±5.24	5.36	8.31	2.18 ±0.2.75	2.05	4.13

Table (2) : Frequency distribution (%) of most identified psychrotrophic bacteria isolated from beef sausage under chilling storage (4°c)

Psychrotrophic Bacteria	Time / Day				
	0	2	4	6	8
Acinetobacter spp.	3 (3.6)	0 (0)	0 (0)	0 (0)	0 (0)
Aeromonas spp.	8 (9.6)	2 (2.7)	0 (0)	0 (0)	0 (0)
Bacillus spp.	15 (18.1)	18 (24.3)	13 (15.1)	9 (10.3)	5 (4.7)
Coliform	4 (4.8)	2 (2.7)	0 (0)	0 (0)	0(0)
Enterobacter spp.	4 (4.8)	5 (6.8)	6 (7)	0 (0)	0 (0)
Lactobacillus spp.	2 (2.4)	6 (8)	19 (22.1)	32 (36.8)	45 (42.1)
Leuconostoc spp.	3 (3.6)	5 (6.8)	16 (18.6)	25 (28.7)	30 (28)
Micrococcus spp.	12 (14.5)	15 (20.3)	11 (12.8)	8 (9.3)	9 (8.4)
Pseudomonas spp.	5 (6)	5 (6.8)	3 (3.5)	1 (1.1)	1 (0.9)
Staphylococcus spp.	21 (25.3)	12 (16.2)	10 (11.6)	2 (2.3)	2 (1.9)
Streptococcus spp.	6 (7.3)	4 (5.4)	8 (9.3)	10 (11.5)	15 (14)
Total	83 (100)	74 (100)	86 (100)	87 (100)	107 (100)

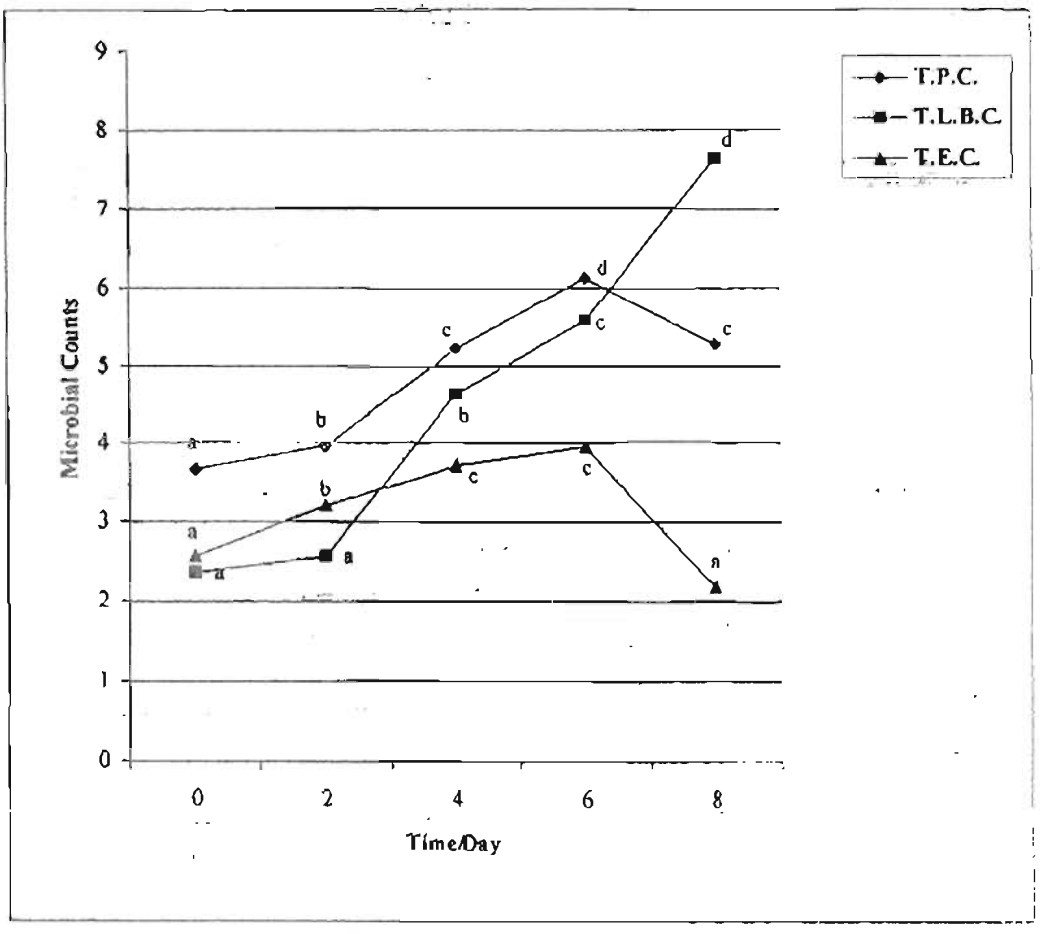
Table (3) : Frequency distribution (%) of most identified lactic acid bacteria isolated from beef sausage under chilling storage (4°c)

Lactic Acid Bacteria	Time / Day				
	0	2	4	6	8
Lactobacillus spp.	10 (34.5)	22 (28.2)	30 (29.4)	48 (35.3)	55 (34.8)
Leuconostoc spp.	8 (27.6)	19 (24.4)	24 (23.5)	32 (23.5)	46 (29.1)
Pediococcus spp.	6 (20.7)	26 (33.3)	32 (31.4)	40 (29.4)	42 (26.6)
Streptococcus spp.	5 (17.2)	11 (14.1)	16 (15.7)	16 (11.8)	15 (9.5)
Total	29 (100)	78 (100)	102 (100)	136 (100)	158 (100)

Table (4) : Frequency distribution (%) of most identified Enterobacteriaceae group isolated from beef sausage under chilling storage (4°c)

Enterobacteriaceae	Time / Day				
	0	2	4	6	8
Citrobacter freundii	16 (16.7)	8 (10.7)	2 (4.9)	5 (15.6)	6 (27.3)
Enterobacter spp.	22 (22.9)	18 (24)	7 (17.1)	7 (21.9)	5 (22.7)
Escherichia coli	13 (13.5)	16 (21.3)	11 (26.8)	8 (25)	2 (9.1)
Hafnia alvei	4 (4.2)	1 (1.3)	0 (0)	0 (0)	0 (0)
Klebsiella spp.	8 (8.3)	3 (4)	0 (0)	0 (0)	0 (0)
Proteus spp.	17 (17.7)	20 (26.7)	21 (51.2)	12 (37.5)	9 (40.9)
Serratia spp.	16 (16.7)	9 (12)	0 (0)	0 (0)	0 (0)
Total	96 (100)	75 (100)	41 (100)	32 (100)	22 (100)

Figure (1) : Changes in log mean values of total psychrotrophic, lactic acid bacteria and enterobacteriaceae counts of chilled beef sausage during chilling storage at 4°c.



Means Log by the same letter in the same line are not significantly different (P < 0.05)
T.P.C. = Total Psychrotrophic count T.L.B. = Total Lactic acid bacteria count
T.E.C. = Total Enterobacteriaceae count

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الملخص العربى

تأثير التبريد على بعض التغيرات البكتريولوچية فى السجق البقرى الطازج

المشتركون فى البحث

على معوض أحمد خالد إبراهيم سلام* مكرم أحمد محمد يس

قسم الرقابة الصحية على الأغذية - كلية الطب البيطرى - جامعة قناة السويس

قسم الرقابة الصحية على الأغذية - كلية الطب البيطرى - جامعة المنصورة*

تم جمع ثلاثون عينة عشوائية من السجق البقرى الطازج والمصنع محلياً من مختلف معارض اللحوم بمدينة الإسماعيلية لدراسة التغيرات البكتريولوچية به تحت ظروف التخزين بالمبرد عند درجة حرارة أربعة درجات مئوية وقد أوضحت الدراسة أن متوسط لوج العدد الكلى للبكتريا المحبة للبرودة هو ٣٫٦٨ و ٣٫٩٥ و ٥٫٢٣ و ٦٫١١ و ٥٫٢٨ ميكروب لكل جرام بينما كان ذلك المتوسط للبكتريا المنتجة لحامض اللاكتيك هو ٢٫٣٦ و ٢٫٥٨ و ٤٫٦٤ و ٥٫٥٧ و ٧٫٦٤ ميكروب لكل جرام ولمجموعة الأنتريوباكتريسى هو ٢٫٥٦ و ٣٫٢٢ و ٣٫٧٣ و ٣٫٩٥ و ٢٫١٧ ميكروب لكل جرام وذلك بعد مرور صفر و ٢ و ٤ و ٦ و ٨ أيام على الترتيب من الحفظ فى المبرد عند درجة حرارة أربعة درجات مئوية، وقد كان هناك تباين معنوياً فى متوسط العدد الكلى للبكتريا المحبة للبرودة عند كل زمن للتحليل الميكروبيولوجى بينما كانت الزيادة غير معنوية بالنسبة للعدد الكلى للبكريا المنتجة لحامض اللاكتيك حتى يومين من التخزين ثم إرتفع العدد معنوياً إلى الحد الأقصى (٧٫٦٤ لوج ميكروب لكل جرام) بعد ثمانية أيام من الحفظ فى المبرد، وعلى الجانب الآخر فإن العدد الكلى لمجموعة الأنتريوباكتريسى إنخفض معنوياً إلى ٢٫١٨ ميكروب لكل جرام فى نهاية فترة التخزين (٨ أيام)، واتضح من الدراسة أن السجق الطازج المصنع محلياً بواسطة القصابون فى مدينة الإسماعيلية يحتاج إلى إتخاذ بعض التقنيات لرفع الجودة وإطالة فترة صلاحيته، هذا وقد ذكر فى هذه الدراسة التوصيات والإقتراحات اللازمة لرفع جودة السجق الطازج المصنع محلياً.