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Effect of Frozen Storage on the Chemical, Physical and Microbiological Quality of imported Mackerel (*Scomber scombrus*).

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ABSTRACT

The imported frozen (Mackerel) *Scomber scombrus* was evaluated for the changes of its quality during freezing storage at -18°C for four months and its compliance with the Egyptian standard specifications. It was found that the protein, lipid and ash contents decreased during the storage period. Furthermore, moisture slightly increased during the storage period. The results also indicated that the samples did not exceed the permissible limits of total nitrogen as determined by the Egyptian Organization for Standardization and the quality of production. Microbiological examination showed a significant decrease [P<0.05] in the total bacterial count between the first and fourth months of storage. *Staphylococcus spp.* was detected in the first and the second months of surface examination, being 104.70 cfu×10³/g and 2.74 cfu×10³/g, respectively. In addition, *Salmonella* was detected in the first month of surface examination. *E. coli* and *Clostridium* were absent in all examined samples. Fourteen different bacterial isolates were isolated from all samples. Twelve isolates were isolated from staph 110 medium and two isolates were found on the SS agar medium. Twelve isolates were coccoid shaped bacteria and 2 isolates were spore forming long rods. All isolates were gram positive. Two isolates were spore forming and twelve isolates were non-spore forming. Six isolates were considered as *Staphylococcus sp.* and another six isolates were considered as *Micrococcus sp.* the last two isolates were considered as *Bacillus sp.*

Keywords: *Scomber scombrus*, Chemical characteristics, Microbiological examinations and Frozen storage.

INTRODUCTION

Egypt is considered the second top ten countries consuming Mackerel (*Scomber scombrus*). Although Egypt came at the first order between Arab countries in fish production, the Egyptian imported fish percentage represented 0.477% in accordance to the world fish production and there were no exported amounts could be significant during the same year (FAO, 2009). Mackerel (*Scomber scombrus*) is a migrant type. Spawning followed by migration of adults belongs to the southern and western areas to feed zones in the Norwegian Sea and the North Sea. This species supports valuable fisheries of great importance in several European countries, with annual mean landings of 675000 Tons from 1972 to 2002 (Dobrinis *et al.*, 2011). Atlantic Mackerel (*Scomber scombrus*) is a worthy specie in the marine industry (Wasik *et al.* 2015). Mackerel fish are important fishery resources contribute to food materials, exceedingly distributed in the Pacific coast of Japan, East China Sea, and zones of Indian (Zhang and Deng, 2012). Fish have many nutrients, so that it is rapid for spoiled foods. Spoilage of fish directly happened after capture. Changes in the fish are done in 2 days by the flesh enzymes. These enzymatic activities decrease because of low temperature where the microflora and the enzymes are not active in these conditions (Gandotra *et al.*, 2012). Preservation of food is necessary to raise its shelf life, protect its nutritional value, flavor and texture. So that, good methods of food preservation must prevent spoilage of food by microorganisms while maintaining its quality and nutritional value (Ghaly *et al.*, 2010). The extension of shelf life can be achieved by freezing, salting, chilling, glazing, smoking, etc. Freezing creates unfavorable conditions which slow down the microbial growth and the biochemical decomposition of fish muscle (Gandotra *et al.*, 2012). To protect the consumer and ensure the quality of fish coming to the Arab Republic of Egypt, the Egyptian Organization for Standardization and Quality status Egyptian standards used by stakeholders to judge the quality of the various imports and foremost of which is frozen fish, which amounted to

Egypt's imports of which 257 thousand tons in 2012 (Hassan *et al.*, 2014). The goals of this study were to: to evaluate the quality of imported frozen Mackerel (Chemical, physical, and microbial examinations) and insure of the compliance with the Egyptian standard specifications. Finally, study the effect of the freezing storage at -18°C on the quality of frozen fish.

MATERIALS AND METHODS

Fish samples collection:

Imported frozen Mackerel (*Scomber scombrus*) was produced at: 10/1/2015, with a period of validity up to six months. Fish samples were collected after entering to New Damietta ports, Egypt. The fish samples directly transported in frozen transport to the laboratory of Food Industries Department, Faculty of Agriculture, Damietta University. The fish sample was divided into 4 groups to evaluate their quality monthly and for four months from the date of production and examine the effect of frozen storage at -18±2 °C for four months and follow up changes occurred during frozen storage at -18±2 °C for four months.

Analytically Methods:

Physical properties:

Water holding capacity (WHC) was determined as described by (Soloviev, 1966), Drip loss was determined as described by (Wangtueai *et al.*, 2014), pH value and acidity were determined using AOAC (2012) methods.

Chemical analysis:

Fish samples were prepared for chemical analysis by removing the viscera, fins, scales, head and bones. The meat is then taken and mixing well according to EOS (2006).

Moisture, protein, fat and ash and non-protein nitrogen content were determination by the method of AOAC (2012). Total volatile basic nitrogen was done by the method described in EOS (2006). Total soluble nitrogen was determined by a method of (Saeed and Howell, 2002). Free amino nitrogen was appreciated by the method of AOAC (2012).

The tested samples were transferred from food industries laboratory to the microbiology laboratory into the ice box. The microbiological examination of fish samples were done in two fish parts (surface and muscle). The microbiological examination of surface was determined according to the method of El-Kadi and El-Morsy (2016). Twenty ml of sterilized buffer phosphate was aseptically transferred to each fish in a new plastic bag. The samples were shaken well for 2 min. The solution was transferred into 30 ml sterilized water and serial dilutions from 10⁻¹ to 10⁻⁵ were done. Defrosting the sample in laboratory temperature (from -18°C to 27°C), Then sterilize the skin with 70% alcohol and a sterile scalpel remove the skin, with new sterile scalpel ten grams of dorsal muscle were taken and aseptically transferred to 90 ml of sterile water. The suspension was shaken for 5 minutes to prepare the first dilution ISO (2003). Three dilutions from 10⁻³ to 10⁻⁵ were done.

Bacterial counting and determination:

Total bacterial of examined fish samples (surface or muscle) were counted on Nutrient agar medium Ronald (2010). Detection of coliform group was done in two stages, the first step is to detect the presence of acid and gas "presumptive test" using MacConkey broth at 37°C for 24-48 hours Ronald (2010). Coliform counts were calculated from standard table according to FDA (1992). Staphylococci were determined as described in APHA (1998) using Staph No. 110 medium. The growing colonies had yellow zones, flat with 1.2 mm diameters were recorded as Staphylococci. Dilution frequency technique was adopted to determine the count of anaerobic spore forming clostridia, using Cooked Meat Medium (CMM). The inoculated tubes were sealed with sterile mixture of Vaseline and Paraffin oil in 1:1 ratio (Vaspar layer) and incubated at 37°C for up to 7 days. The presence of clostridia was detected at the end of the incubation period by accumulation of gases pushing the vaspar layer up using MPN method AOAC (2012).

Isolation and Identification:

One ml of suitable dilution was put into Petri dish, and then melted suitable medium was poured and mixed well then left to solidify. Petri dishes were then incubated at the suitable temperature for appropriate period and obtained developed single microbial colonies of different morphologies in all used cultivation media were picked-up. Colonies were transferred into suitable media plates for sub culturing to obtain pure microbial isolates El-Fadaly et al. (2016).

The obtained bacterial isolates were maintained on suitable media slants at 5°C until use. The identification of the obtained bacterial isolates was done according to Holt et al. (1994) based on the shape, arrangement of the cells, Gram reaction, spore staining and acid fast stain.

Statistical analysis:

Data observed were subjected to a one-way ANOVA statistical method (P<0.05) to explore differences as a result of the previous parameters by using the SPSS statically program for windows version 20, significant differences (P<0.05) between means were determined by Duncan's multiple range test Garcia-Soto et al. (2015).

RESULTS AND DISCUSSION

Physical properties changes of frozen Mackerel during the storage at -18±2°C for four months showed in Table (1). From tabulated data drip loss percentages increased during the storage period from 1.74 ± 0.169% in the first month of the storage to 2.01 ± 0.022% in the fourth month of the storage period. However, there are significant differences [P<0.05] in the drip loss percentages between the first and fourth months of the storage period. The change of myofibril structure, alteration in protein conformation and water retention site in muscles, all of them play an important role in the rise of drip loss Truong et al. (2016). These results agreed with Akter et al. (2014).

As shown in Table 1, the percentage of water holding capacity (WHC) decreased from 58.23±0.666% to 55.38±0.291% during the storage period. Furthermore, there are significant differences [P < 0.05] between the first and fourth months of the storage period in WHC percentages. The decrease in WHC% may be due to biochemical changes in fish muscle such as protein denaturation and lipid oxidation. Furthermore, microbial and enzymatic activities Nguyen et al. (2013). These results agreed with those of Osorio et al. (2015).

Table 1. Physical properties changes of frozen Mackerel during the storage at -18±2°C for four months

Storage period (month)	Physical properties			
	DL%	WHC%	pH	Acidity%
1	1.74 ^b ± 0.169	58.23 ^a ± 0.666	5.96 ^b ± 0.033	1.75 ^a ± 0.057
2	1.82 ^{ab} ± 0.089	56.83 ^{ab} ± 0.641	5.98 ^b ± 0.088	1.72 ^a ± 0.177
3	1.88 ^{ab} ± 0.155	56.76 ^{ab} ± 0.596	6.10 ^{ab} ± 0.082	1.61 ^{ab} ± 0.151
4	2.01 ^a ± 0.022	55.38 ^b ± 0.291	6.20 ^a ± 0.149	1.56 ^b ± 0.033

Preceding data in Table 1 showing that there were significant differences [P<0.05] in pH values of first and fourth months of the storage period. However, values of pH increased from 5.96 ± 0.033 in the first month to 6.20 ± 0.149 in the fourth month of storage period. This rise in pH value can be attributed to the increase of decomposition products such as volatile bases (NH₃, TMA and DMA) and accumulation of amines and peptides Oucif et al. (2012). These results are in agreement with those of Mazrouh (2015). Finally, results in Table 1 showed that, the percentages of acidity decreased during the storage period. Additionally, there were significant differences [P<0.05] between the first and fourth months of the storage period. However, acidity percentages in frozen Mackerel varied from 1.75 ± 0.057% to 1.56 ± 0.033%. These results are consistent with the results recorded by El-Dengawy et al. (2012).

Gross chemical composition:

Approximate composition of frozen Mackerel during the storage at -18±2°C for four months are shown in Table 2. Results showed that, there was slight rise in moisture content from 71.98 ± 0.483% to 72.84 ± 0.220% during the storage at -18±2°C for four months. However, there were no significant differences [P<0.05] among the storage time. These results may be due to reactivity between formaldehyde or malanoaldehyde (breakdown product of trimethylamin (TMA) and total lipid) and tissue protein with liberation of water, rise forming of TMA and TVB result to rise in moisture and lack of protein and lipid

content. These results are in accordance with those of Mohamad and saad (2015).

As shown in Table 2 there was no significant [P<0.05] decrease in protein content. However, it decreased from 68.35 ± 0.471 to 68.12 ± 0.583 during the storage period. These results can be attributed to the effect of freezing and autolytic deterioration connecting with the activity of endogenous enzymes and bacteria during the storage Aberoumand (2013). Similar results were observed by Mazrouh (2015) who found that protein content in *Saurida undosquamis* decrease from 23.12±1.99 to 13.46±1.32 after 7 and 21 days of storage at -20°C, respectively.

Table 2. Approximate composition of frozen Mackerel during the storage at -18±2°C for four months

Storage period/ (month)	Approximate composition			
	Moisture %	Protein %DB	Fat % DB	Ash % DB
1	71.98 ^a ±0.483	68.35 ^a ±0.471	28.49 ^a ±0.412	3.14 ^a ±0.127
2	72.22 ^a ±0.626	68.31 ^a ±0.418	28.47 ^a ±0.490	3.10 ^a ±0.138
3	72.40 ^a ±0.635	68.22 ^a ±0.723	28.43 ^a ±0.497	3.05 ^a ±0.206
4	72.84 ^a ±0.220	68.12 ^a ±0.583	28.40 ^a ±0.589	2.99 ^a ±0.158

Mean ± SD the small letters in the same column means no sig. diff.

There was no significant decrease in fat content during storage of frozen Mackerel at -18±2°C for four months. However, the highest value of fat content 28.49 ± 0.412% was observed in the first month and the least 28.40 ± 0.589% was recorded in the fourth month of the storage period. This decrease may be due to unsaturated fatty acids oxidation and enzymatic hydrolysis in fish and triglyceride fraction loss Mazrouh (2015). Similar results were observed by Alam *et al.* (2012) and Mazrouh (2015).

Ash content in selected fish samples slightly decreased during the storage period from 3.14 ± 0.127% to 2.99 ± 0.158%. However, there were no significant differences in the ash content among the storage period. Those results may be due to muscle content of minerals, physiological parameter and alimentation Mazrouh (2015). These results agreed with those of Roopma *et al.* (2013).

From previous results, it could be concluded that, the nutritional value of fish in terms of the content of protein, fat and ash are very important for human health, not just its suitability for human consumption. Therefore, it is recommend to modify them to the Egyptian standard specifications by including these parameters during fish examination.

Protein fractions:

Table 3 showed protein fractions of frozen Mackerel during the storage at -18 ± 2° C for four months.

Results in Table 3 showing that, there were significant differences [P<0.05] between the first and fourth months in the percentages of the total soluble nitrogen (TSN). Which decreased from 4.94 ± 0.131% to 4.60 ± 0.122% during the storage period. These decrease might be due to protein denaturation and aggregation or fat oxidation products and there interaction with decomposition products of trimethyl amine oxide Alinasabhematabadi (2015). These results are in agreement with those published by Moawad *et al.* (2013).

Non-protein nitrogen % (NPN) raised from 0.271 ± 0.024 in the first month of storage to 0.315 ± 0.018 in the end of the storage period. Furthermore, there were significant differences [P<0.05] in the percentage of NPN%

between the first and fourth months of the storage period. This increase of NPN% may be due to activity of proteolytic enzymes and bacteria (Ramesh *et al.*, 2013). Similar results were obtained by Ramesh *et al.* (2013).

Table 3. Protein fractions of frozen Mackerel during the storage at -18±2°C for four months

Storage period/ (month)	Protein fractions			
	TSN %	NPN %	FAN %	TVB-N mgN/100g
1	4.94 ^a ±0.131	0.271 ^b ±0.024	0.238 ^b ±0.008	19.26 ^b ±0.842
2	4.80 ^{ab} ±0.216	0.287 ^{ab} ±0.013	0.255 ^{ab} ±0.007	20.65 ^{ab} ±0.789
3	4.74 ^{ab} ±0.282	0.308 ^a ±0.040	0.273 ^a ±0.007	21.09 ^a ±0.171
4	4.60 ^b ±0.122	0.315 ^a ±0.018	0.275 ^a ±0.040	21.89 ^a ±0.098

Mean ± SD the small letters in the same column means no sig. diff.

Data in Table 3 showing that, free amino nitrogen (FAN) % increased during the storage period from (0.238 ± 0.008%) to (0.275 ± 0.040%). Furthermore, there were significant differences [P < 0.05] in FAN% between the first and fourth months of the storage time. Those results may be due to the high activity of bacteria and of proteolytic enzymes Ramesh *et al.* (2013). Similar results were recorded by Ramesh *et al.* (2013).

Recorded data in Table 3 reflected that, there were significant differences [P < 0.05] in TVB-N content between the first and fourth months of the storage period. Furthermore, the least value of TVB-N in frozen Mackerel 19.26 ± 0.842 mgN/100g flesh was observed in the first month of the storage while the highest value 21.89 ± 0.098 mgN/100g flesh was recorded in the fourth month of the storage. However, this results are in the permissible limit (Total volatile basic nitrogen of frozen fish must be not more than 30 mg N/100g sample) which recommended by EOS (2009). In general, TVB-N percentage increased with prolong of the storage period. These results may be due to the action of bacteria and endogenous enzymes Hassanin and El-Daly (2013). Similar results were obtained by Paola and Isabel (2015).

Bacteriological examination of frozen Mackerel during the storage at -18±2°C for four months.

Table 4 showing the total bacterial count in surface of frozen Mackerel during the storage at -18±2°C for four months.

Table 4. Surface bacteriological examination of frozen Mackerel during the storage at -18±2°C for four months

Storage period/ (month)	Surface examination (cfu ×10 ³ /g)		
	Total Bacterial Count	<i>Salmonella</i> and <i>Shigella spp.</i> Count	<i>Staphylococci spp.</i> Counts
1	102.88 ^a ±0.500	3.17 ^a ±0.061	104.70 ^a ±0.556
2	99.52 ^{ab} ±0.502	ND*	2.74 ^b ±0.004
3	77.06 ^b ±0.601	ND	ND
4	46.56 ^c ±0.800	ND	ND

*ND: means not detected, Mean ± SD. The smaller letters in the same column means no sig. diff.

Data in Table 4 showed that, surface total bacterial counts were 102.88 ± 0.500, 99.52 ± 0.502, 77.06 ± 0.601 and 46.56 ± 0.800 (cfu×103/g) in 1st, 2nd, 3rd and 4th month, respectively. Furthermore, there were significant differences [P<0.05] between the first and fourth months of the storage period. However, total bacterial count decreased during the storage of Mackerel at -18±2°C for four months. These decreases can be attributed to the frozen storage effect (Mahmoud, 2004). The data in the present study are in accordance with those published by Al-Harbi and Uddin (2005).

The human bacterial pathogens *Salmonella* spp and *Shigella* spp were detected only in the first month of storage period with total count of 3.17 ± 0.061 cfu $\times 10^3$ /g. These results are in agreement with those of Tayo *et al.* (2012b). *Escherichia coli* was not found among the storage at $-18 \pm 2^\circ\text{C}$ for the examined four months. Whereas, *Staphylococcus* spp. was only detected in the first and second months of the storage being 104.70 ± 0.556 cfu $\times 10^3$ /g, 2.74 ± 0.004 cfu $\times 10^3$ /g in the first and second months, respectively, and not detected in the third and fourth months of the storage period. Appearance of *Salmonella* spp and *Shigella* spp and *Staphylococcus* spp may be due to the fish handling with contaminated hands and bacteria could be found on skin, chitinous shell (Emikpe *et al.*, 2011). These results agreed with those of Tayo *et al.* (2012a). Finally, *Clostridium* spp was not detected on surface of the selected fish during the storage at $-18 \pm 2^\circ\text{C}$ for the examined four months. Similar results were obtained by Rokibul *et al.* (2013).

Dorsal muscle bacteriological examination:

Table 5 showing total bacterial counts in the dorsal muscle of frozen Mackerel during the storage at $-18 \pm 2^\circ\text{C}$ for four months. Results in Table 5 indicated that, total bacterial count decreased during the storage time from 6.15 ± 0.042 cfu $\times 10^3$ /g in the first month to 3.30 ± 0.078 cfu $\times 10^3$ /g in the fourth month of storage time. Furthermore, there were significant differences [$P < 0.05$] between the first and fourth months. The present data are in accordance with those published by Mahmoud (2004).

Table 5. Dorsal muscle bacteriological examination of frozen Mackerel during the storage at $-18 \pm 2^\circ\text{C}$ for four months

Storage period/ (month)	Dorsal muscle examination (cfu $\times 10^3$ /g) TBC
1	$6.15^a \pm 0.042$
2	$6.00^a \pm 0.052$
3	$4.35^b \pm 0.055$
4	$3.30^b \pm 0.078$

Mean \pm SD. The smaller letters in the same column means no sig. diff.

The decrease in total bacterial count during storage period can be attributed to freezing which is known to reduce viable cell counts by 1-2 Log units, with the increase of storage time, and the reduction in viable cell was increased Oraei *et al.* (2011). *Salmonella* sp., *Shigella* sp., *E. coli*, *Staphylococcus* sp. and *Clostridium* sp. bacteria were not detected in all samples during the storage. These results are in agreement with those by Mahmoud (2004) and Phadke *et al.* (2012).

These results can be due to that, most pathogens don't multiply at temperature of freezer and many of them die because their enzymes don't work properly to maintain normal cell activity. As well, pathogens need water to grow and freezing turns the available water into ice crystals Oranusi *et al.* (2014). Furthermore, the absence of *E. coli* may be attributed to that samples not contaminated with fecal or may be due to frozen storage effect as reported by Phadke *et al.* (2012).

From these results of total bacterial count of dorsal and surface in Mackerel during the storage period, it could be concluded that, the total bacterial counts in the surface of all samples were higher than that found in dorsal muscle of the same samples. These results can be due to the skin, improves the growth of both anaerobic and aerobic bacteria

(Tayo *et al.*, 2009). These results are in agreement with that found by Tayo *et al.* (2012a) and Sichewo *et al.* (2014).

Characterization and identification of bacterial isolates:

Fourteen bacterial isolates were obtained from all fish samples. Twelve different bacterial isolates were isolated from the staph. 110 medium (Nos. 1, 2, 3, 4, 5, 6, 7, 8, 11, 12, 13 and 14) and two isolates from SS agar medium (Nos. 9 and 10). The characterization of bacterial isolates were as following; 12 isolates were cocci and 2 isolates were long rods. All isolates were Gram positive. 2 isolates were spore formers and 12 isolates were non spore formers.

Typical colonies representing *Staphylococci* growth, creamy or orange color colonies were picked up and streaked onto the same specific media slant. After growth, spherical cells, Gram positive, arranged in irregular clusters. Isolates Nos. 3, 5, 7, 8, 11 and 14 were considered as *Staphylococcus* sp. according to Bergey's Manual of Determinative Bacteriology Holt *et al.* (1994). Isolates Nos. 1, 4, 9, 10, 12 and 13 were considered as *Micrococcus* sp. These results are consistent with that obtained by Eze *et al.* (2011) who isolate *Staphylococcus aureus* from frozen Mackerel fish (*Scomber scombrus*). Also, Odebiyi *et al.* (2013) isolated *Staphylococcus aureus*, *Staphylococcus epididemis* and *Micrococcus* sp. from the fish samples.

Colonies which were white, yellow or orange were picked up and streaked onto the same specific media slant. After growth, the cells were long rods, Gram positive and spore forming. Its arrangement were single, pair or chains. Isolates Nos. 2 and 6 were considered as *Bacillus* sp. according to Bergey's Manual of Determinative Bacteriology Holt *et al.* (1994). Similar results were obtained by Odebiyi *et al.* (2013) who isolated and identified *Bacillus subtilis* from the fish samples.

CONCLUSION

Finally, it could be concluded that, the nutritional value of fish in terms of the content of protein, fat and ash are very important to human health, not just its suitability for human consumption. Therefore, it is recommend adding them to the Egyptian standard specifications examine fish. Frozen Mackerel samples had dorsal total bacterial counts less than the permissible limit of the Egyptian Organization for Standardization (EOS) and these samples are accepted according to the EOS.

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تأثير التخزين بالتجميد على الجودة الكيميائية، الفيزيائية والميكروبيولوجية لسماك الماكريل (سكومبر سكومبروس) المستورد.

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تم تقييم التغيرات التي تحدث في سمك الماكريل المجمد والمستورد أثناء تخزينه بالتجميد عند درجة حرارة -18°م لمدة أربعة أشهر وتحديد مدي مطابقته للمواصفات القياسية المصرية. وقد أظهرت النتائج انخفاض في نسبة البروتين والدهن والرماد انخفاضاً غير معنوي خلال فترة التخزين بينما زادت نسبة الرطوبة زيادة غير معنوية خلال فترة التخزين. كما أشارت النتائج إلي عدم تجاوز العينات الحدود المسموح بها من النيتروجين المتطاير الكلي كما قررت الهيئة المصرية للتوحيد القياسي وجودة الإنتاج. كما سجلت نتائج الفحص الميكروبيولوجي انخفاضاً معنوياً في العدد الكلي للبكتريا. وكان عدد بكتريا *Staphylococcus* في الشهر الأول والثاني 1.0×10^4 و 1.0×10^2 (خلية بكتيرية/سم^٢) على الترتيب. ظهرت بكتريا *Salmonella* في الشهر الأول فقط على السطح الخارجي للسمك، بينما لم تظهر كلا من بكتريا *E. coli* و *Clostridium* أثناء فترة التخزين. تم عزل ١٤ عزلة بكتيرية، ١٢ عزلة من علي بيئة ال staph و عزلتين من علي بيئة ال SS. ١٢ عزلة كروية الشكل و عزلتين لها شكل العصوي الطويل. كانت كل العزلات موجبة لجرام. وعزلتين مكونة للجراثيم الداخلية و ١٢ عزلة غير متجرتمة، ٦ عزلات تم تعريفها علي أنها بكتريا ال *Staphylococcus* sp. وعرفت ٦ عزلات اخري علي أنها بكتريا ال *Micrococcus* sp. بينما العزلتين الأخرتين تم تعريفها علي أنها بكتريا ال *Bacillus* sp.