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Effect of Vacuum Packaging Technique, Refrigeration and Freezing on Beef Quality

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Abstract

The present study was designed to investigate the influence of vacuum packaging on chemical and microbial parameters of beef during frozen and refrigeration storage. Quality assessment of vacuum packaged beef up to 10 weeks was done by the monitoring of quality, TVB-N, free fatty acids (FFA), thiobarbituric acid (TBA), Histamine, pH and some bacterial count such as Enterobacteriaceae count,Psychrotrophic counts,Mesophilic count and lactic acid bacteria. A total of 100 fresh beef samples,20 samples of them were examined biochemically and bacteriologically on zero day before starting the experiment and the rest of 80 samples were divided into two main groups, 40 samples each. First main group divided into two subgroups, 20 samples each. First subgroup (A) stored in freezer without vacuum packaging for ten weeks at -18°C. The second subgroup (A) were vacuumed byVacsy system and kept in freezer for ten weeks at -18°C. The second main group was divided into two subgroup, 20 samples each. First subgroup (B) stored on shelf of refrigerator without vacuum packaging for 5 days at 4°C. The second subgroup(B) were vacuumed by Vacsy system and stored in cyst of vacuum then all samples of the second main group were kept on shelf of refrigerator for 5 days at 4°C. 4 samples were taken from each subgroup of the first main group (A) every two weeks until the end of the experiment(ten weeks),also 4 samples were taken from the second main group (B) daily for 5 days (4 samples from each subgroup) and subjected to biochemical and bacteriological examination. Obtained results showed that TVB-N, (FFA), (TBA), Histamine and pH values of vacuum packaging samples were significantly lower than those in non-vacuum packaging samples. Results indicated that vacuum packaging was effective in reduce lipid oxidation and increased shelf life of beef. Similarly the microbial load of vacuum packaging samples were significantly lower as compared to non-vacuum packaging samples. Results also indicated that vacuum packaging had a very good effect on the organoleptic properties such as color, odor and taste during refrigeration, but their was no clear effect during freezing period on the organoleptic properties. Thus, the employment of vacuum packaging alone or in combination with other protective strategies is recommended.

Key wards : vacuum packaging, beef, frozen and refrigeration.

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Introduction

Vacuum packaging of fresh meat has proved to be efficient in extending shelf life, preserving the sensory characteristics inherent to the product for a period sufficiently long. During refrigeration, the vacuum allows the shelf life of chilled meat to be extended by reducing oxidation and the growth of aerobic bacteria. Of the established meat packaging systems, the vacuum has been the most widely used in the institutional market for the distribution of whole pieces. In North America, approximately 85% of fresh meat and most processed meats are packed in a modified atmosphere, including the vacuum system**(Ercolini *et al.*,2009).** Vacuum packing has become popular as a protection technique during refrigeration. Shelf life quality of food products can be improved by vacuum packing technique. Moreover, the microbial ecology of food basically depends on the environment, used equipment, food type, handling practices, processing, packaging and storage temperature**(Sachindra, 2005).**

For strict anaerobic microorganisms, such as certain *Clostridium* species, cultivation in conventional food microbiology laboratories is difficult because it requires highly reduced mediums and the use of special equipment and systems with strict anaerobiosis..The initial contamination of meat occurs during bleeding, due to the use of non-sterile equipment, and introduces microorganisms into the vascular system. Meat is a semi-solid medium that is relatively low in sugar, which acts as a good media for feeding bacteria **(Labadie 1999).**

Vacuum packaging, which is used in the conditioning of whole pieces or small parts, aims to protect the meat from contact with oxygen from the air. Oxygen promotes the growth of aerobic microorganisms, which can change the odor, color and appearance of meat products, leading to oxidative rancidity of the fats, change the meat pigments and destroy vitamins and flavors **(Sarantópoulos and Soler, 1991).**

Vacuum-packaged meats are generally quite stable at low temperature **(Labadie, 1999**).

Fresh meat products are commonly marketed at refrigerated temperature (2–5°C). However, many undesirable changes of the products can occur during refrigeration due to microbial growth and lipid oxidation, which give rise to quality reduction, meat spoilage, and economic loss **(Sallam and Samejimab, 2007) .**

Minimizing product contamination and delaying or inhibiting growth of spoilage and pathogenic organisms in the product are major keys for improving fresh meat shelf life and increasing consumer safety. While general cleanliness and proper sanitation are very effective, other means of controlling microbial growth in meat products may be prove useful. Total volatile basic nitrogen (TVB-N) is known as a

product of bacterial spoilage and endogenous enzymes action and its content is often used as an index to assess the keeping quality and shelf-life of products **(European CommissionEC, 1995).**

(TVB-N), are produced by bacterial action and generate changes in texture, color, odor and flavor **(Li *et al*., 2011)**. These changes can be classified as biochemical, physical and microbiological, and determine the degree of acceptance by consumers and, along with nutritional evaluation, shelf life **(McMillin, 2008).**

Therefore the main objective of this study was to observe biochemical and microbial changes in meat under vacuum-packaging technique and without packaging technique in frozen meat without blast frozen and chilled meat.

Material and Methods

Samples of fresh beef were collected randomly from the markets in Dakahlia Governorate from muscles of thigh and immediately transported to the laboratory and examined at the day of purchasing. Total 100 fresh beef samples,20 samples aut of which, were examined biochemically and bacteriologically on zero day before starting the experiment and the remained 80 samples were divided into two main groups, (40samples each). First main group (A)divided into two subgroups, 20samples each. First subgroup (A) stored frozen without vacuum packaging for 10 weeks at -18°C. The second subgroup (A)were vacuumed byVacsy system and stored in cyst of vacuum then the samples were kept in freezer for 10 weeks at -18°C. The second main group was divided into two subgroup, 20samples each. First subgroup (B) stored on shelf of refrigerator without vacuum in normal cystfor 5 days at 4 °C. The second subgroup(B) were vacuumed by Vacsy system and in cyst of vacuum then all samples of the second main group were kept on shelf of refrigerator for 5 days at 4 °C.4 samples were taken from each subgroup of the first main group (A) every two weeks until the end of the experiment(ten weeks),also 4samples were taken from the second main group (B)daily for 5 days (4 samples from each subgroup). All samples were transferred in ice box immediately to the laboratory under complete aseptic precautions without delay and were subjected to the following examination, determination of total volatile basic nitrogen (TVBN) mg/100g according to **(Association of Official Analytical Chemists AOAC (2000).**

Determination of thiobarbituric acid (TBA) mgDM|Kg according to **Association of Official Analytical Chemists AOAC (2000).**

Free fatty acid analysis (FFA), expressed as % of oleic acid, was determined according to **Association of Official Analytical Chemists AOAC (2000).**

- Quantitative Assessment of Histamine was done by using thin layer chromatography method**,** according to **(INFO SAMAK 1989).**

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The PH of meat was determined by the method of**(Association of Official Analytical Chemists AOAC 2000).**

Bacteriological Examination

Preparation of samples for bacteriological examination

25g of meat samples were homogenized in 225 ml of sterile buffered peptone water for 1 min using a Stomacher 400 Lab Blender (Seward Medical, London, UK). Decimal serial dilutions were made in the same sterile peptone water and used for microbiological analyses of the beef samples at each of the appropriate time intervals during refrigerated and frozen storage according to **Iso 6887-2 (2003).**

Enterobacteriaceae count: Were conducted according to**:**

(APHA 2001).

Mesophilic and psychrophilic

Mesophilic and psychrophilic microorganism counts were conducted according to **(*ISO 2004*)**

Lactic acid bacteria :According to method conducted by **Food and Agriculture Organization FAO (1980).**

Organoleptic evaluation :

The organoleptic properties of samples were evaluated by five trained panelists on a 10-point scale for assessment of odor, taste, color and slime formation according to **Sundrma and Urbai (1992).**

Statistical analysis : Statistical analyses were performed using SPSS statistical program (version 2000 for Windows, SPSS Inc., Chicago, IL, USA). (2000).

Results and Discussion

For fresh meat, vacuum packaging has proved to be efficient in extending shelf life, preserving the sensory characteristics inherent to the product for a period sufficiently long for its turnover. During refrigeration, the vacuum allows the shelf life of the meat to be extended by reducing oxidation and the growth of aerobic microorganisms of the established meat packaging systems, the vacuum has been the most widely used in the institutional market for the distribution of whole pieces **(Maria *et al*.,2011).**

Obtained result, in the present study showed that TVB-N in zero day group were (7.3 ± 0.8 mg|100g) as shown in table (1) but first subgroup (A) which preserved in refrigerator without vacuum after 10weeks were (19.83± 1.7mg|100 g.) as shown in table (2), while the second subgroup (A) which vacuumed byVacsy system and preserved in cyst of vacuum followed by refrigeration for ten weeks

were (17.52 ±1.5mg|100g). Table (2) also showed that first subgroup (B) which preserved on shelf of refrigerator without vacuum for 5 days were (25.64± 1.90 mg|100g) which exceeded the suggestive limits (20mg|100g) recommended by the ***(Egyptian Standard Specification"ESS" 1990)***.

Concerning to the second subgroup (B) which vacuumed byVacsy system and preserved in cyst of vacuum then kept on shelf of refrigerator for 5 days TVB-N were (20.06± 1.8mg|100g). (TVB-N), which are produced by bacterial action and generate changes in texture, color, odor and flavor (Li et al., 2011). These changes can be classified as biochemical and microbiological, and determine the degree of acceptance by consumers and, along with nutritional valuation, shelf life ***(McMillin, 2008)***.

This behavior is attributed to decreased autolytic activity and the beginning of the microbial degradation process, which is common in all meats***(Li et al., 2011)***.Our results were in agree with **(Maria *et al*.,2011).**

Tabel (1) showed that TBA in zero day were (0.17±0.02mg DM|Kg, but table (3) showed that TBA in the first subgroup(A) after 10 weeks were (0.923±0.07mg DM|Kg), TBA in the second subgroup (A) after 10 weeks were (0.831±0.05 mg DM|Kg), TBA in the first subgroup (B) after 5 days were (2.354±0.17 mg DM|Kg), TBA in The second subgroup (B) after 5days were (1.01±0.09 mg DM|Kg) which exceeded the permissible limit (0.9 mg DM|K) according to**(Egyptian Standard Specification"ESS" 1990).** Thiobarbituric acid (TBA) value is an index which measures the malondialdehyde (MDA) content and is a widely used method for assessment of degree of lipid oxidation. MDA is formed through hydroperoxides, which are the initial reaction products of polyunsaturated fatty acids with oxygen. The present study showed a progressive increase in TBA value (secondary oxidation product) with increase in storage period under frozen conditions. The values rose from 0.17±0.02 on day zero to 0.923±0.07 in first subgroup (A) after ten weeks and 0.831 ±0.05 mg DM/kg in vacuum packaging in the second subgroup (A) after frozen storage period, our results were in agree with**(Mohammed *et al*., 2014 and Vieira *et al*. 2009)**.

Vacuum packaging has been found to substantially reduce oxidative deterioration in frozen meat. TBA was highly rised in the first subgroup (B) were (2.354± 0.17 mg DM|Kg), which preserved on shelf of refrigerator without vacuum and exceeded the permissible limit (0.9 mg DM|K) according to ***(Egyptian Standard Specification"ESS" 1990),*** our results were in agree with ***Roopma et al.,(2015).***

Table (1) also showed that Free fatty acid were in zero day group (0.215± 0.02 g| 100g) and rose in table 4 in first subgroup (A) after ten weeks to be (0.974± 0.08 g|100g) , in the second subgroup (A) after ten weeks were (0.865± 0.07g|100g)

, while highly significant increase in the first subgroup (B) were (2.463± 0.15 g|100g) and in the second subgroup(B) were (0.912± 0.08 g|100g).The results thus clearly depicts, that there was a gradual increase in the FFA content with increasing storage time. The levels had also direct correlation with TBA(table 3) showing that it could act as a good indicator for the assessment of the freshness of meat .***Balev et al. (2011)*** reported that at the end of storage the total FFA concentration in air packaged is higher than vacuum packaging.

Histamine as shown in table (1) in zero day were (0.08 ± 0.006 mg|Kg), in table 5 in first subgroup(A) after 10 weeks were(0.37±0.02mg|kg), insecond subgroup (A) after 10 weeks were (0.232 ± 0.03mg|Kg), in first subgroup (B) after 5days were(0.366± 0.04 mg|Kg) , in second subgroup (B) after 5days were (0.311± 0.03mg|kg) under vacuum. Histamine formation can be decreased by refigerated storage under vacuum and histamine amount depend on the production date and increase by closing to expiration date , our results were in agree with ***(Claudia, and Francisco 2004)***

pH: The pH values also showed an increasing trend with increase in frozen period. The pH values were 5 .8 ±0.4on day zero and rose in first subgroup (A) after 10 weeks to be(6.2± 0. 5) , in the second subgroup (A) after 10 weeks were (6.1± 0.5) , while significant increase in the first subgroup (B) were (6.7± 0.05) which exceed the permissible

limit(5.6-6.2) according to **International Standard Organization ISO (1979)** and in the second subgroup(B) were (6± 0.3). Decrease or constant levels of pH in the second subgroup (A) might be attributed to increasing solubility of CO2 at storage time, effecting on growth of microflora,**Taheri and Motallaabi (2012).**

Enterobacteriaceae in zero day were (1.3± 0.1) log10cfu/g,as shown in table(1), but in first subgroup (A) after 10 weeks were (1.98± 0.2) log10cfu/g,as shown in table(7), in second subgroup (A) after 10 weeks were (1.9±0.2) log10cfu/g, in first subgroup (B) were (2.23± 0.2) log10cfu/g, which exceeded the maximal recommended limit of 2 log10 cfu/g for Enterobacteriaceae in meat ***(International Commission on Microbiological Specification for Foods ''ICMSF''1986***, in second subgroup (B) were (1.94±0.2) log10cfu/g. Enterobacteriaceae can multiply in vacuum-packaged meat causing deterioration and pack distension at refrigeration temperature. These microorganisms have been the subject of many studies. That are able to grow at refrigeration temperatures have been identified as causative agents of blowing vacuum packages ***Brightwell (2007).*** The presence of Enterobacteriaceae in vacuum-packed chilled meat are of particular importance, both for its high deteriorating potential and for food safety concerns because some species are

pathogens. Recent research in New Zealandon blown packs of chilled fresh meat stored at refrigeration detected a moderate to high number of Enterobacteriaceae**.**

Psychrotrophic in zero day were (1.6 ±0.8) log_{10}cfu/gas shown in table (1), but in first subgroup (A) after 10 weeks were (4.22± 0.4)log_{10}cfu/gas shown in table (8), in second subgroup (A) after 10 weeks were (3.94 ±0.3),), in first subgroup (B) after5days were (5.52 ± 0.5)log_{10}cfu/gwhich exceeded the maximal recommended limit of 5 log_{10} cfu/g for*Psychrotrophic* in meat **(International Commission on Microbiological Specification for Foods ‘‘ICMSF‘‘1986)**, in second subgroup(B) were (4.32±0.4***)*** log_{10}cfu/gas shown in table (8) **.** In vacuum-packaged meat, *psychrotrophic* facultative anaerobic and anaerobic bacteria can grow and cause different types of spoilage ***Ray and Bhunia (2008)***.

Typically, *mesophilic* bacteria are those that dominate the initial microbiota of vacuum-packaged meat ***Ray and Bhunia (2008)***. The deterioration caused by *psychrotrophic* is associated with proteolysis, loss of texture, accumulation of liquid in packages and an unpleasant smell, mainly hydrogen sulfide gas. In anaerobic conditions, proteins are degraded into sulfur compounds, which have strong and disgusting odor. End products of non-protein nitrogen compounds generally include ammonia ***Ray and Bhunia (2008).***

Mesophilic bacteria in zero day were (1.3±0.06) log_{10}cfu/g as shown in table(1), but in first subgroup (A)after ten weeks were (4.41 ± 0.4)as shown in table(9), but, in second subgroup (A) after ten weeks were (3.75±0.3) log_{10}cfu/g, in first subgroup (B) after 5days without vacuum were (5.02± 0.5) log_{10}cfu/g, which exceeded the maximal recommended limit of 5 log_{10} cfu/g for *Mesophilic* bacteria in meat **(International Commission on Microbiological Specification for Foods ‘‘ICMSF‘‘1986)**, in second subgroup (B) with vacuum were (4.17±0.4 ***)***as shown in table (9),our results were agree with ***Maria et al., (2011)***.

Lactic acid bacteria

in zero day were (1.7±0.09) log_{10}cfu/gas shown in table(1), but in first subgroup (A) after 10weeks were(4.67± 0.4) as shown in table(10), in second subgroup (A) after ten weeks were (4.52±0.4), in first subgroup (B) after ten weeks were (5.01 ± 0.5), in second subgroup (B) were(4.76±0.4) log_{10}cfu/gas shown in table(10).Storage conditions usually favor the growth of lactic acid bacteria.deterioration caused by species of Lactic acid bacteria is not regarded as particularly undesirable because the odor of the volatile fatty acids that are produced by these microorganisms disappears after opening the package ***Signorini et al. 2006***.Even recommended the application of some species of lactic bacteria and lactic acid in meat as a means of controlling bacterial populations and increasing shelf life.

When lactic bacteria produce H_2S from cysteine, however, they produce an unpleasant odor and color. H_2S oxidizes myoglobin to metmyoglobin, giving meat a green color. Heterofermentative species produce lactic acid and CO_2, which leads to the accumulation of gas and liquid in the package ***Ray and Bhunia (2008)***.

Lactic acid bacteria causes the accumulation of CO_2 in vacuum-packaged meat.Some lactic acid bacteria are more harmful to the quality of meat than others. During prolonged storage in a modified atmosphere, some heterofermentative strains can produce fermentation products such as butyric acid and ethanol, which reduce the shelf-life of the product ***Holley et al.,(2004)***.

Lactic acid bacteria ferment glucose and other substrates that are present in meat. When these substrates are depleted, growth stops, typically when the population reaches 8 log/cm^2. The metabolic residues of most lactic acid bacteria are not eliminated and can be identified as slightly acidic or milky tastes ***Holley et al.,(2004)***.

Initially lactic acid bacteria were in zero day(1.7±0.09) log cfu/g and increased to(4.67± 0.4) log cfu/g after ten weeks of freezing and lactic acid bacteria were (4.42±0.3), log_{10}cfu/g after vacuum packaging frozen samples at the end of storage and after five days on the shelf were (4.91± 0.5) log_{10}cfu/g. **International Commission on Microbiological Specification for Foods *ICMSF (2005)*** recorded that level of microbial contamination in meat show signs of alteration is 7.0 log cfu/g , in present study as shown in table 10 lactic acid bacteria after 5 days on the shelf under vacuum-packaging technique were (4.66 ± 0.4)) The complete removal of oxygen from meat ensures longer preservation against microbial deterioration than packaging in oxygen **Iacurto et al.,2005**.Table (11),(12) showed that the results of an evaluation of the organoleptic properties of the examined samples showed that the values of the analyzed parameters varied slightly over storage period and also showed that vacuum-packaging technique had a very good effect on the organoleptic properties of beef.TVB-N, which are produced by bacterial action generate changes in texture, color, odor and flavor ***(Li et al., 2011).***our results were in agree with **Iacurtoet al., 2005 and Soldatou et al., (2009).**

Conclusion

The freezing of meat at low temperature makes it less prone to spoilage by decreasing the bacterial activity. However, it was observed that an increasingin biochemical composition and microbial count during frozen storage. Therefore, it could be concluded that freezing with vacuum packaging technique is the best preferred when preservation of meat is priority.

Vacuum packages for meat increase the shelf life and thus improve the distribution efficiency and marketing of meat. Deterioration problems are minimized

when the pH of the meat to be packaged is controlled and ideal storage temperature are accurately maintained. vacuum-packaging technique could be utilized successfully to maintain chemical quality, reduce the microbial growth, and extend the shelf life of meat during refrigerated storage.

Vacuum packaging technique has been found to substantially reduce oxidative deterioration in frozen meat.

Table(1) Mean value of biochemical and bacteriological parameters in examined samples in zero day group

Group Parameters	***Zero day group***	***Bacterial count***	***Zero day group mean ±SE $log_{10}cfu/g$***
TVB-Nm g|100g	7.3 ± 0.8	Enterobacteriacae	1.3± 0.1
TBA mgDM|Kg	0.17 ± 0.02	Psychrotrophic	1.6 ± 0.08
Free Fatty Acid g|100g	0.215 ± 0.02	Mesophilic	1.3 ± 0.06
Histamine mg|100g	0.8 ± 0.06	Aerobic plate count	1.7 ± 0.09
PH	5.8± 0.4	Lactic acid bacteria	1.5 ± 0.06

Table(2) Mean value of TVB-N (mg|100g) in different group samples. (n=20 of each).

Group / Time	**First main group**		**Group / Time**	**Second main group**	
	First subgroup (A)	Second subgroup (A)		First subgroup (B)	Second subgroup (B)
2 weeks	11.56±0.47	9.11±0.41*	1day	8.7±0.42	7.82±0.22*
4 weeks	14±0.66	12.07±0.48*	2days	13.6±0.53	10.74±0.3**
6 weeks	16.58±0.39	13.61±0.7**	3days	17.55±0.57	15.21±0.49*
8 weeks	18.75±0.42	15.32±0.4**	4days	20.45±0.62	18.31±0.52*
10 weeks	19.38± 0.48	17.52±0.46*	5days	25.04±0.72	20.6±0.57**

P* < 0.05, p < 0.01 by using t-test.**

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Table(3) Mean value of TBA (mgDM |Kg) in different group samples. (n=20 of each).

Group / ***Time***	**First main group**		***Group*** / ***Time***	**Second main group**	
	First subgroup(A)	Second subgroup(A)	***1day***	First subgroup (B)	Second subgroup (B)
2 weeks	0.354±0.04	0.207±0.02*		0.227±0.03	0.193±0.02
4 weeks	0.552 ± 0.05	0.419± 0.04*	2days	0.508± 0.04	0.322± 0.04*
6 weeks	0.724± 0.05	0.583± 0.03*	3days	0.725± 0.06	0.514± 0.05*
8 weeks	0.859± 0.04	0.712±0.04*	4days	1.372± 0.09	0.834± 0.06**
10 weeks	0.923± 0.07	0.831± 0.7	5days	2.354± 0.11	1.01±0.07**

P* < 0.05, p < 0.01 by using t-test.**

Table(4)Mean value of Free Fatty Acid (g| 100g) in different group samples. (n=20 of each).

Group Time	**First main group**		**Group Time**	**Second main group**	
	Firs tsubgroup (A)	Second subgroup(A)	***1day***	First subgroup (B)	Second subgroup (B)
2 weeks	0.383±0.03	0.288±0.02*		0.354±0.03	0.235±0.02*
***4* weeks**	0.581± 0.03	0.463± 0.03*	2days	0.570± 0.03	0.356±0.02**
6 weeks	0.781± 0.05	0.542± 0.04**	3days	0.753± 0.04	0.531± 0.03**
8 weeks	0.894± 0.04	0.775±0.03*	4days	0.912± 0.05	0.724± 0.04*
10 weeks	0.974± 0.04	0.825± 0.04*	5days	1.463±0.08	0.912±0.08***

P* < 0.05, p < 0.01, p*** < 0.001 by using t-test**

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Table(5) Mean value of Histamine (mg|kg) in different group samples. (n=20 of each).

Group / Time	**First main group**		**Group** / **Time**	**Second main group**	
	First subgroup (A)	Second subgroup(A)	1day	First subgroup (B)	Second subgroup (B)
2 weeks	0.183±0.02.	0.115±0.01*		0.105±0.01	0.092±0.008
4 weeks	0.245± 0.02	0.186± 0.02	2days	0.205± 0.01	0.162± 0.02
***6* weeks**	0.287± 0.02	0.209±0.01*	3days	0.285± 0.02	0.182± 0.02*
8 weeks	0.328± 0.03	0.282±0.02	4days	0.421± 0..03	0.325± 0.02*
10 weeks	0.370± 0.03	0.232±0.03*	5days	0.366± 0.03	0.311±0.02

P*< 0.05 by using t-test.

Table(6) Mean value of PH in different group samples. (n=20 of each).

Group / ***Time***	**First main group**		**Group** / **Time**	**Second main group**	
	First subgroup (A)	Second subgroup(A)	***1day***	First subgroup (B)	Second subgroup (B)
2 weeks	5.8±0.04	5.7±0.04		5.8±0.04	5.7±0.04
4 weeks	6.±0.05	5.8± 0. 04*	2days	5.9.± 0.04	5.8± 0.04
6 weeks	6.1± 0.04	5.9± 0.05*	3days	6± 0.05	5.9± 0.04
8 weeks	6.2± 0.05	6.±0.04*	4days	6.3±0.05	6.1± 0.04*
10 weeks	6.2± 0.05	6.1± 0.04	5days	6.7±0. 05	6.2±0.05**

P*< 0.05, p< 0.01 by using t-test.**

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Table (7) Mean value of Enterobacteriaceae in in different group samples, mean ±SE log_{10}cfu/g (n=20 of each).

Group Time	**First main group**		**Group Time**	**Second main group**	
	First subgroup (A)	Second subgroup (A)	1day	First subgroup (B)	Second subgroup (B)
2 weeks	1.63± 0.18	1.54± 0.2		1.7±0.2	1.49±0.21
4 weeks	1.88± 0.2	1.62±0.19	2days	1.87±0 .2	1.65± 0.18
6 weeks	1.91± 0.2	1.82±0.2	3days	1.93± 0.2	1.76± 0.2
8 weeks	1.95± 0.2	1.86± 0.2	4days	1.98±0.2	1.88± 0.2
10 weeks	1.98± 0.2	1.90± 0.2	5days	2.23± 0.2	1.94±0.2

Table(8) Mean value of Psychrotrophic bacteria in different group samples, mean ±SE log_{10}cfu/g (n=20 of each).

Group Time	**First main Group**		**Group Time**	**Second main group**	
	First subgroup(A)	Second subgroup(A)	1day	First subgroup (B)	Second subgroup (B)
2 weeks	2.02±0.2	1.91± 0.2		1.90± 0.2	1.82±0.19
4 weeks	2.84 ± 0.2	2.49 ±0.2	2days	2.94 ±0 .3	2.43± 0.2
6 weeks	3.62± 0.3	3.15±0.2	3days	3.77± 0.4	3.69± 0.2
8 weeks	4.01± 0.4	3.85± 0.3	4days	4.35± 0.5	4.11± 0.4
10 weeks	4.22 ±0.4	3.94± 0.3	5days	5.25±0.5	4.32±0.4

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Table(9) Mean value of Mesophilic bacteria in different group samples , mean ±SE log_{10}cfu/g .(n= 20 of each).

Group / ***Time***	**First maingroup**		**Group Time**	**Second main group**	
	First subgroup (A)	Second subgroup (A)	1day	First subgroup (B)	Second subgroup (B)
2 weeks	1.91± 0.2	1.79± 0.2		1.84± 0.2	1.66±0.18
4 weeks	2.63± 0.2	2.29±0.2	2days	2.72± 0 .2	2.52± 0.2
6 weeks	3.05± 0.2	2.87±0.2	3days	3.26± 0.3	3.02±0.2
8 weeks	3.84± 0.4	3.57± 0.3	4days	4.13± 0.5	3.62± 0.4
10 weeks	4.41±0.4	3.75± 0.3	5days	5.02±0.5	4.17±0.4

Table (10)Mean valueofLactic acid bacteria in different group samples, mean ±SE log_{10}cfu/g (n=20 of each).

Group / Time	**First maingroup**		**Group Time**	**Second main group**	
	First subgroup (A)	Second subgroup (A)	1day	First subgroup (B)	Second subgroup (B)
2 weeks	2.23± 0.2	1.88± 0.2		1.91± 0.2	1.71±0.18
4 weeks	2.72± 0.2	2.19±0.2	2days	2.68± 0 .2	2.01± 0.19
6 weeks	3.92± 0.3	3.22±0.2	3days	4.12± 0.3	3.53± 0.2
8 weeks	4.45± 0.4	3.93± 0.3	4days	4.66± 0.4	4.07± 0.3
10 weeks	4.67± 0.4	4.42±0.4	5days	4.91± 0.5	4.66± 0.4

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Table (11) Effect of refrigeration on the organoleptic properties of examined samples of 2nd group during storage at 4°C

Without vacuum					With vacuum				
Days	Oder	Taste	color	Slime formation	Days	Oder	Taste	Color	Slime formation
0 day	9.6	9.7	9.6	ND	0days	-	-	-	-
1 day	9.3	9.5	9.4	ND	1 days	9.5	9.7	9.5	ND
2	9.1	9.2	9.3	ND	2 days	9.4	9.5	9.4	ND
3 days	8.9	8.8	9	ND	3 days	9.	9.1	9.2	ND
4 days	6.9	6.7	6.5	ND	4 days	8.7	8.6	8.5	ND
5 days	4.8	4.6	4.2	ND	5 days	7.9	7.8	7.5	ND

ND = not detected

Table (12) Effect of freezing storage at - 18°C on the organoleptic properties of examined samples

Without vacuum					with vacuum				
weeks	Odor	Taste	color	Slime formation	weeks	Odor	Taste	Color	Slime formation
2 weeks	9.4	9.6	9.4	ND	2 weeks	9.5	9.5	9.5	ND
4 weeks	9.3	9.5	9.4	ND	4 weeks	9.5	9.4	9.5	ND
6 weeks	9.1	9	9.1	ND	6 weeks	9.2	9.1	9.3	ND
8 weeks	8.5	8.4	8.3	ND	8 weeks	9	8.7	8.5	ND
10 weeks	7.8	7.7	7.4	ND	10 weeks	8.3	8.5	8.1	ND

ND = not detected

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