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INVESTIGATING BACTERIAL CONTAMINATION IN ICE CREAM OFFERED IN LOCAL MARKETS (LOCAL AND IMPORTED)

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ABSTRACT

The research study included the investigation of microbial contamination of ice cream samples that packed automatically in specialized factories, both local and imported available in the markets of Baghdad City, a number of 10 samples were taken from of July to August 2022, which is the peak season of its production, using the laboratory method used in microbial examinations comparing it with one of the modern technologies represented by the BacTrac 4300 technology, the study showed that there is a convergence in the results of the two methods used, as the total number of aerobic bacteria Total plate count, Total Colifrom bacteria, and *Staphylococcus aureus* bacteria are higher than the permissible limits in Iraqi standard specification for dairy and grafted ice cream No. (702) for the year 1984. While the study samples were devoid of *Salmonella* spp. bacteria.

Keywords: Ice cream, microbial contamination, BacTrac 4300 , local markets

التحري عن التلوث البكتيري في الأيس كريم المعروض في الأسواق المحلية (محلي ومستورد)

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الخلاصة

تضمنت دراسة البحث التحري عن مدى التلوث الميكروبي لعينات المثلجات اللبنية المعبئة آليا في مصانع متخصصة لذلك بنوعيها المحلية والمستوردة المتوافرة في اسواق مدينة بغداد، وبعدد 10عينات لكل منهما خلال شهري تموز واب للعام 2022 والذي يعد موسم ذروة انتاجها، باستعمال الطريقة المختبرية المتبعة في الفحوصات الميكروبية ومقارنتها بإحدى التقانات الحديثة المتمثلة بجهاز BacTrac 4300، أظهرت الدراسة ان هنالك تقارب في نتائج الطريقتين المتبعتين، اذ ان العدد الكلي للبكتريا الهوائية (Total plate count) وبكتريا القولون (Total Coli From Bacteria) وبكتريا المكورات العنقودية الذهبية *Staphylococcus aurous* اعلى من الحدود المسموح بها في المواصفة القياسية العراقية للمثلجات اللبنية والمطعمة رقم (702) لسنة 1984. بينما خلت عينات الدراسة من بكتريا *Salmonella spp.*.

الكلمات المفتاحية: الأيس كريم، التلوث الميكروبي، جهاز BacTrac 4300، الاسواق المحلية.

INTRODUCTION

Milk ice cream is considered one of the food and consumer items in general as a food commodity, especially in the summer season, because of its positive effect in softening body temperature, in addition to being an energy-generating substance **(Atil *et al.*, 2011).** As well as its nutritional importance for what it contains in the components of its raw material, and it can be defined as a mixture that can be obtained by freezing a mixture consisting of various ingredients (pasteurized milk, cream, sugar, flavorings, natural and artificial colourings,

stabilizers, emulsifiers and other permitted additives. It is believed that the Chinese the first person to innovate it by manufacturing a mixture of ice and fruit juice with the aim of serving it as a dish after food, as he does not know the exact period of time in which the manufacture of this milky ice cream appeared. It is believed that the Chinese is first person to innovate it by manufacturing a mixture of ice and fruit juice with the aim of serving it as a dish after food, as it is not known exactly the time period in which the manufacture of this milky ice cream appeared (**AL-Timimi *et al.*, 2012**). And because the nature of its components and the health conditions from which it is made have contributed greatly to the quality of the product because of its suitability for the activity of many microorganisms, including bacteria, and thisis what studies have indicated, as the starting point of manufacturing depends on what the raw material (milk) contains of bacterial content, being an appropriate medium For the growth of many pathogenic and foodborne microorganisms, as well as the people in charge of the manufacturing process by not following health conditions such as personal hygiene while doing work, as well as the tools and machines used in manufacturing, which are a suitable medium for the rapid growth and reproduction of microorganisms **(Al-Musawi *et al.*, 2017).** Storage conditions are one of the factors affecting the limitation of bacterial growth, as storage must be at temperatures below zero Celsius, this is confirmed through his study **Babid *et al.*, (2018).** Bacterial growth is more dangerous for survival, growth and rapid reproduction at temperatures ranging between (2.5-4)°C, this degree will enhance the increase in the number of its cells in contaminated food, especially when preserved for a long time, as it affects different age groups in general and children in particular, as well About its harmful effect on pregnant women, due to its direct relationship with cases of miscarriage, premature birth, and meningitis in newborn (**Jong *et al.*, 2020**). In view of the specificity of this product in Iraq, which is consumed throughout the year in general and in the summer in particular, if we know that it is made either in a traditional way that lacks the most basic requirements of health conditions, in addition to selling it in the markets in a way that is not subject to health control, or it is done in an automated way and packaged in specialized factories so Hence this study, which aimed to investigate some microbial contaminants in the manufactured and automatically packaged ice cream product that is presented in the local markets in the City of Baghdad, both local and imported, using the laboratory method used in microbial examinations, and comparing it with one of the modern technologies represented by BacTrac 4300 technology, which is characterized by its high accuracy, as well as its short time in executing the examination process

MATERIALS AND METHODS

Collection of samples:

The process was carried out on samples of ice cream, both local and imported, available in the markets of the City of Baghdad, randomly, at the rate of 10 samples each during July to August of year 2022, which is the peak season of its production. They were transported to the laboratory in a refrigerated container prepared for this purpose, after placing the study samples in polyethylene plastic bags. Each of them was placed separately in sterile plastic bags until arriving at the laboratory.

Preparation of samples

The method described by (**Pagotto *et al*. 2001)** was followed in preparing samples for testing by heated them at room temperature and under sterile conditions, transferring 11 milliliters of each sample under sterile conditions to the envelope of the Stomacher mixer after adding an appropriate amount of liquid enrichment medium, pre-sterilized peptone solution and subject In glass vials at a rate of 99 milliliters, then the sample was mixed at a speed of 2000 revolutions/minute for two minutes, after that the mixture was added to the rest of the contents of the sterile vial containing the enrichment medium to be incubated in the refrigerator at a temperature of 8 °C for a period of 30 minutes, and these are the most important steps, this is the first dilution 10^{-1} and I completed the rest of the decimal dilutions by adding 1 ml of the first dilution to 9 ml of the peptone solution, and so I completed the rest of the dilutions until the 10^{-6} dilution, then the following tests were performed.:-

Microbial examination

The microbial examination of the studied samples was estimated according to (**A.O.A.C., 1998)** and included:-

Total bacterial count

Nutrient agar was used 1 ml of each dilution was transferred to a Petri dish separately by a sterile pipette, then the medium was poured after cooling it to a temperature of 45 °C then dishes were stirred gently for homogenization and distribution well, left to solidify the dishes were turned over and incubated at a temperature of 37°C for 24 h, then the Count the number of colonies developing in the dishes.

Total Coliform Bacteria

Violet Red Bile Agar medium (V.R.B.A) was used to estimate the number of coliform bacteria, transferring 1 ml of the appropriate dilution into sterilized Petri dishes, then pouring the medium into the dishes until it hardens, then adding another layer of the same culture medium, in order to provide anaerobic conditions. It was incubated at a temperature of 37°C for 24 h. After the end of the incubation period, the growing colonies, which represent the number of coliform bacteria, were counted.

Staphylococcus aurous

0.1 ml of pre-prepared dilutions was spread to the medium of Mannitol salt agar using L-shape media separately. They were incubated at 37 °C for 24 hours, and the number of colonies growing as *Staphylococcus aureus* in the dishes was counted after the end of the incubation period using the coagulase test. To distinguish between *Staphylococcus aureus* and other types of *Staphylococcus* bacteria.

Salmonella spp.

The initial activation was carried out by using the enriched Nutrient Broth , this step is a means to activate the bacteria in the sample, if found after incubating it for 24 hours at a temperature of 37 °C. Transferring 1 ml of the medium into tubes containing 9 ml of Selenite Broth – Trathiouate broth, the aim of this process It is the elimination of intestinal bacteria, with the exception of Salmonella bacteria. It was incubated at a temperature of 37 °C for 24 hours, then by means of a loop from both media, then transferred to *salmonella shigella* agar, which is one of the differential media for *Salmonella* bacteria.

Using bactrac 4300 technology

All microbiological examinations were carried out with a BacTrac 4300 according to **ISO FDIS (2015**). Which included the total count of aerobic bacteria using (BiMedia 001 B), prepared by the company SY-LAB Austrian, as the required amount of the medium was dissolved in distilled water and 9 ml were placed in each tube, with the temperature of 121°C and a pressure of 15 pounds / inch2 for 15 minutes. As for *E.coli* bacteria, the medium (BiMedia 150 B) was used, that dissolved in distilled water, the pH value of the medium was adjusted to 6.80 and placed in an autoclave for sterilization, then it was cooled to a temperature of less than 45°C, then placed In Measuring cell by 9 ml in each previously sterilized tube, as for *Staphylococcus aurous* bacteria. The medium was used (BiMedia 350 A) that dissolved in distilled water, the pH value of the medium was adjusted to 7.00, placed in a bunker for sterilization then cooled to a temperature less than 37°C, placed in a measuring cell of 9 ml in each previously sterilized tube, the presence of bacteria was detected. *Salmonella* spp. in the samples using the medium BiMedia 205A Base and mixed with a certain amount of distilled water A BiMedia 205A A ditive according to the instructions of the supplying company, then distributed in the pre-sterilized glass bottles of 9 ml and placed in a water bath at a temperature of 60 °C for 10 minutes.

RESULTS AND DISCUSSION

The results of microbial examinations of local and imported ice cream samples under study in the city of Baghdad in Table,1 showed that the average number of aerobic bacteria count ranged between (1×10^2 - 6×10^6) CFU/g. These results coincided with the findings of (**Rossi *et al*., 2017; Abid *et al*., 2018**), as the total number of bacteria count ranged from 2.41×10^3 to 7.62×10^4 CFU/g. And when compared the results reached by each of **Gorgy *et al*.,(2016) and Bolaji *et al*., (2018),** we find that the number of aerobic bacteria were did not exceed $2x10^3$ CFU/g, compared to the results achieved in the research under study, which exceeded the permissible limits in Iraqi Standard No. (702) for the year 1984. While for the numbers of coliform bacteria for samples under this study, the rate ranged between (1×10^2 - 7×10^3) CFU/g, these results are close to found by (**Davin *et al*., 2019**) and (**Abid *et al*., 2018**), coliform bacteria ranged between (2.17×10^3 to 6.9×10^6) and ($2.45x10^2$ to $8.77x10^3$) CFU/g, respectively, while it was higher than the permissible limits in the Iraqi standard referred to above, the reason for the existence of these numbers of bacteria lead to post-pasteurization contamination, as well as the raw materials involved in the manufacture of ice cream, manufacturing methods and their circulation, which leads to contamination with large numbers of coliform, as well as storage methods and conditions of changing temperatures and humidity that make them suitable conditions for the growth and reproduction of microorganisms. reference as for the numbers of *Staphylococcus aureus* bacteria ranged between ($2 x10^2$-$3x10^3$) CFU/g, which is higher than the permissible limits in the Iraqi Standard Specification **IQS 702 (1980),** the cause of contamination with this type of bacteria is either through contact with the contaminated hands of workers, as they are present in a form it may be natural on the skin and hands of people or milk pasteurization process may not be sufficient to eliminate it if the growth stops for a period of time and then it grows and reproduces when suitable conditions for growth and reproduction are available or it may return to the materials added to the ice cream mixture, or as a result of transporting and handling samples and storage temperatures higher than 4°C, and the results showed that all samples were clear from *Salmonella* spp.

Table(1): Microbial examination of ice cream samples local and imported (CFU/g).

N	Types	Total plate count	Total coliform	*Staphylococcs aurous*	*Salmonella*
1	Local	1×10^2	2×10^2	3×10^2	-
	Imported	1×10^3	7×10^3	1×10^3	-
2	Local	5×10^2	1×10^2	2×10^2	-
	Imported	7×10^4	3×10^3	3×10^3	-
3	Local	5×10^2	2×10^2	6×10^2	-
	Imported	6×10^6	7×10^3	1×10^3	-
4	Local	4×10^2	1×10^2	3×10^2	-
	Imported	5×10^4	3×10^2	1×10^3	-
5	Local	2×10^4	2×10^2	2×10^2	-
	Imported	4×10^4	6×10^3	3×10^2	-
6	Local	3×10^2	2×10^2	6×10^2	-
	Imported	7×10^5	1×10^3	1×10^3	-
7	Local	6×10^2	1×10^2	3×10^2	-
	Imported	2×10^3	5×10^3	1×10^3	-
8	Local	6×10^3	3×10^2	2×10^2	-
	Imported	4×10^4	1×10^3	3×10^3	-
9	Local	4×10^2	6×10^2	6×10^2	-
	Imported	5×10^6	2×10^3	1×10^3	-
10	Local	3×10^2	1×10^2	3×10^2	-
	Imported	4×10^3	3×10^3	1×10^3	-

Microbial examinations by using BacTrac 4300 device:

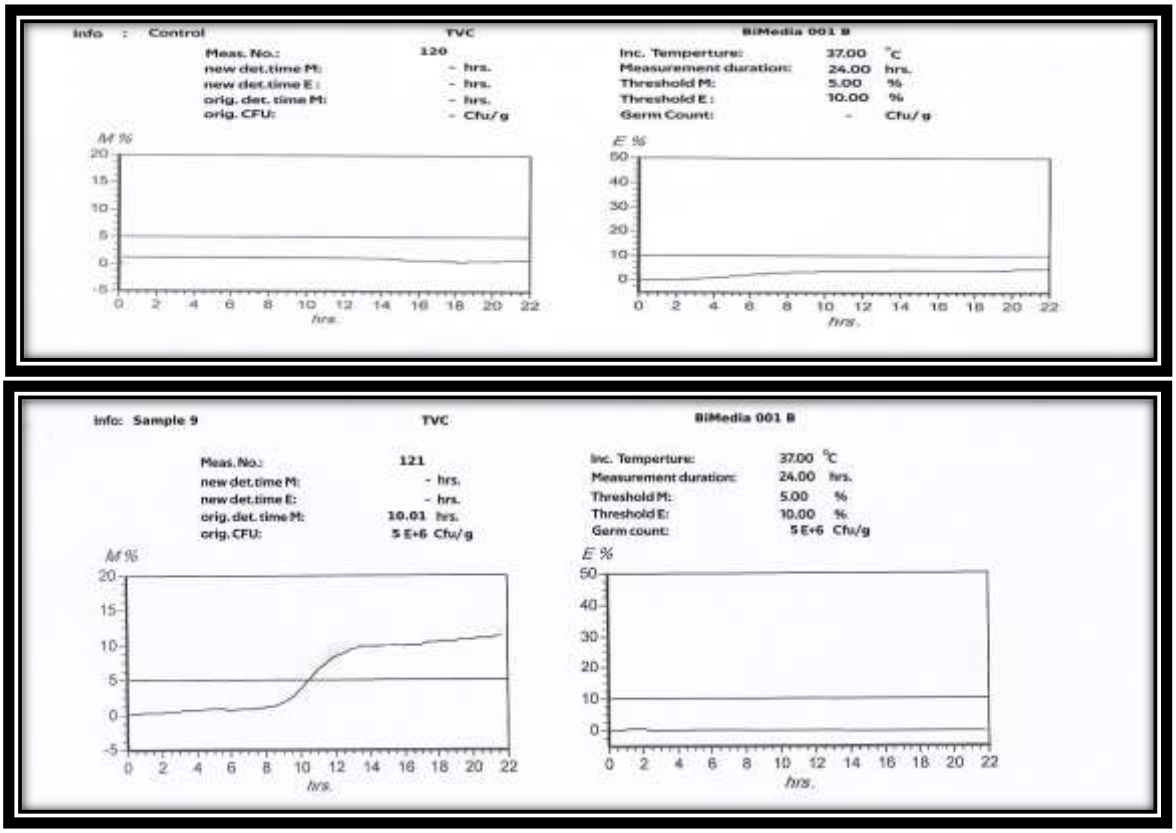
The results of microbial examinations of ice cream samples using the BacTrac4300 device (Table,2), showed that the total count of aerobic bacteria ranged between (1×10^2 - 5×10^6) CFU/g , and this result agreement with the results reported by **Nwinyi *et al.*, (2017**) in the results of the total count of aerobic bacteria, which amounted to 2.5×10^4 CFU/g, using the BacTrac 4300, one of the important characteristics of the BacTrac device that it determines the time of starting of growth for bacteria through a graphic curve as shown in (Figure,1), as the curve shows The growth began after 10 hours of incubation in all samples. As for coliform bacteria, the numbers ranged between (1×10^2 - 6×10^3) CFU/g, and the growth time appeared 12 hours from the beginning of incubation, as shown in (Figure,2). Approach to the results obtained in the traditional microbial examination of the ice cream samples under study, this was confirmed by **Samir *et al.*(2018).** When examining the ice cream samples, the number of

coliform bacteria reached 3.7 x 10^3 CFU/g in the ice cream samples, the number of *Staphylococcus* bacteria reached the lowest value in sample No.1, which amounted to 1 x 10^2 CFU/g, and the highest value in the sample No.8, which amounted to 4 ×10^5 CFU /g, the results were close to what appeared in the traditional microbial examination in this study with what **Hussein, (2015)** found in ice cream products, as it ranged between ($1.2×10^2$ -$6.1×10^2$) CFU/g, and the results showed that the local and imported ice cream samples were clear from the presence of salmonella bacteria, as shown in (Figure, 3).

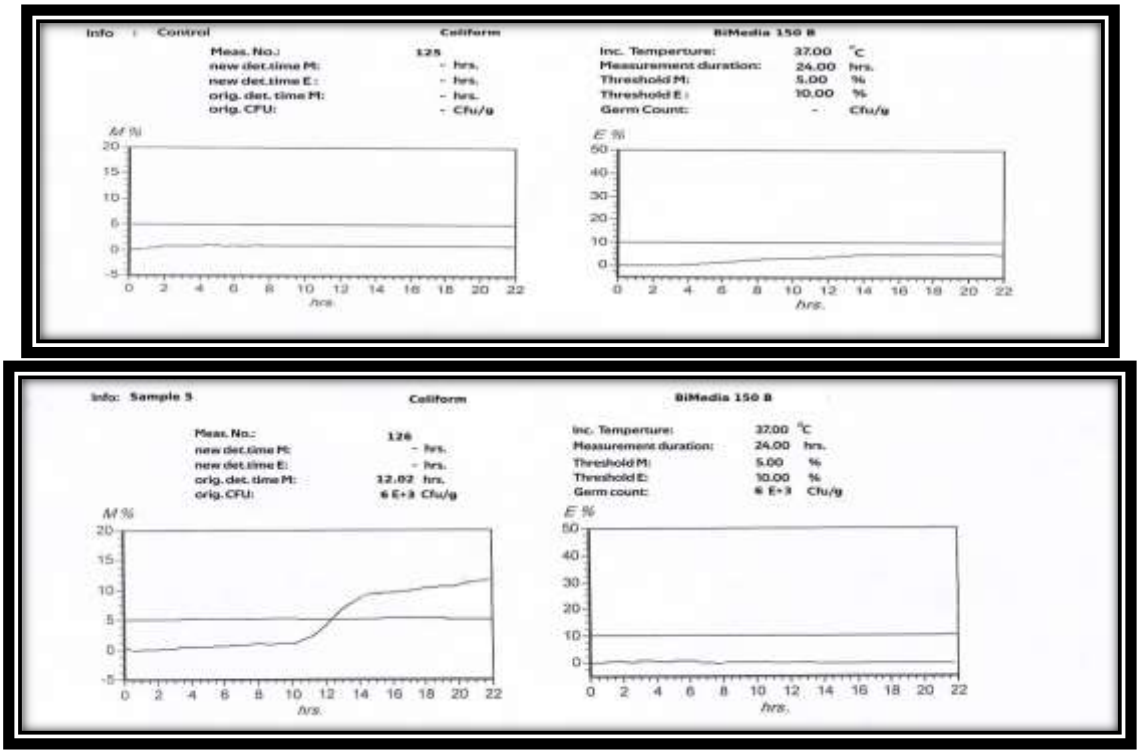
In view of the lack of health safety to preserve this material, it is exposed to bacterial contamination arising from the surrounding environmental conditions, as well as the unhealthy way of dealing, which includes the method of manufacturing, processing and packaging, as well as the nature of the raw materials involved in the manufacturing process, the cleanliness of the machines, the conditions of storage and transportation, and the safety of workers in this sector, which is considered one of the most important factors influencing its preservation in a fresh and safe condition for the health of the consumer, and attention to factories through tightening health control and conducting laboratory tests for the final product and strict adherence to protection and safety laws by conducting periodic checks for workers inside the factory, in order to prevent the transmission of bacteria from workers to the manufactured ice cream, taking care of the factory environment, sterilizing devices, equipment, machines, and collection tanks on an ongoing basis, and taking care to store products in frozen at a temperature below zero degrees Celsius in order to prevent bacterial growth, and taking care to use raw materials that are not contaminated or expired, and not using preservatives that affect consumer health and interest in setting expiration dates for manufactured and worn out products Familiar with storage conditions and conducting periodic visits by the state's regulatory authorities to ice cream factories for the purpose of inspection and control of the production process and the correct detection of the level of safety inside the factories, and the use of modern technologies in conducting microbial tests such as BacTrac 4300, which is characterized by high accuracy and a short time and effort in giving results, while conducting microbial examinations by traditional methods requires time and effort, the results appear less accurate **(Zina, 2014**). The modern technology, represented by the BacTrac 4300, provides additional data that researchers can benefit from, it is the complete diagram of the growth phase of microorganisms, as it determines the start time of the first growth of the organism. Microscopy, time of highest growth and between stages with high accuracy.

Table (2): Results of microbial examinations by using the BacTrac 4300**.**

N	Types	Total plate count	Total coliform	*Staphylococcs aurous*	*Salmonella*
1	Local	1×10^2	4×10^2	1×10^2	-
	Imported	3×10^5	2×10^3	6×10^3	-
2	Local	2×10^2	3×10^2	4×10^2	-
	Imported	3×10^3	4×10^3	1×10^5	-
3	Local	3×10^2	1×10^2	3×10^2	-
	Imported	6×10^4	6×10^2	2×10^5	-
4	Local	1×10^2	9×10^2	1×10^2	-
	Imported	2×10^3	2×10^3	6×10^3	
5	Local	2×10^2	3×10^2	8×10^2	-
	Imported	3×10^6	4×10^3	2×10^5	-
6	Local	4×10^2	2×10^2	2×10^3	-
	Imported	6×10^2	6×10^3	5×10^4	-
7	Local	1×10^2	1×10^2	3×10^2	-
	Imported	5×10^6	2×10^2	6×10^3	-
8	Local	2×10^2	3×10^2	5×10^2	-
	Imported	3×10^2	4×10^3	4×10^5	-
9	Local	4×10^2	1×10^2	2×10^2	-
	Imported	6×10^4	4×10^2	3×10^5	-
10	Local	1×10^2	2×10^2	6×10^3	-
	Imported	2.1×10^4	3×10^3	2×10^4	-



Figure(1): shows control and Curve growth of Bacteria using Bimedia 001B with Bactrac 4300methods for 24 hrs.



Figure(2): shows control and Curve growth of coliform Bacteria using Bimedia 150B with Bactrac 4300methods for 24 hrs.

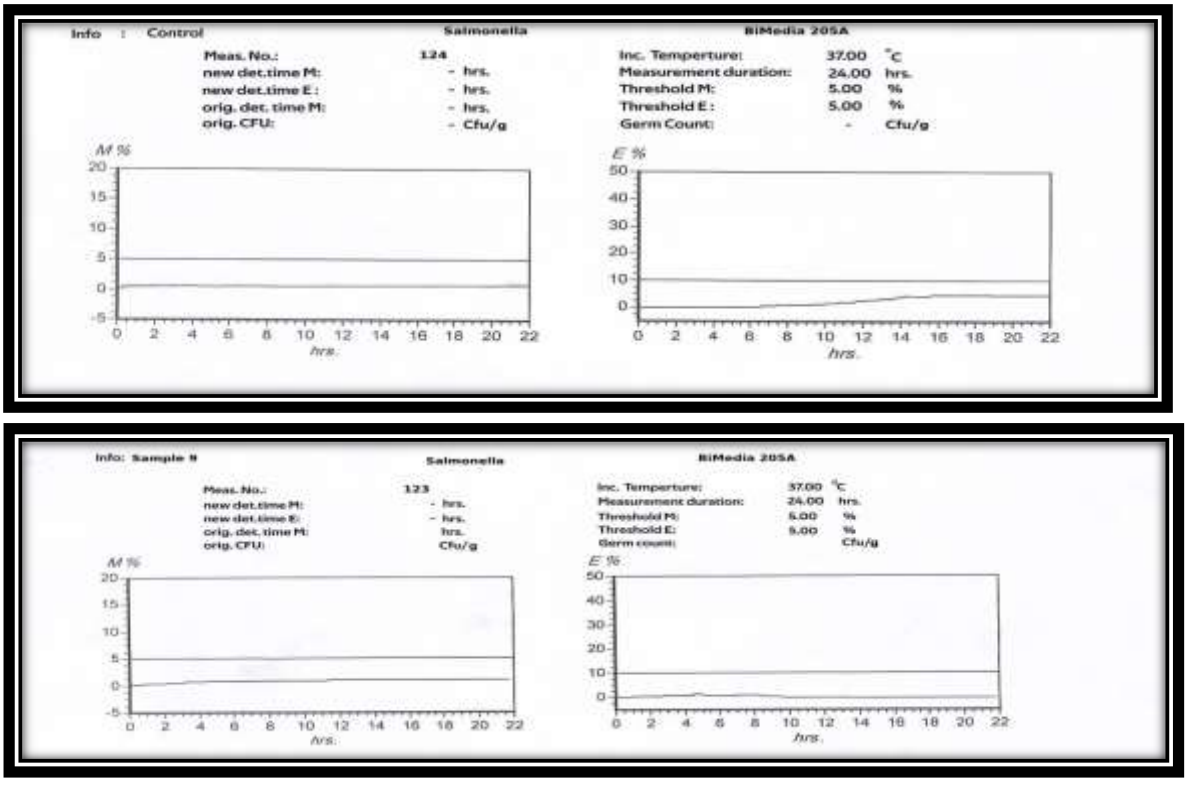


Figure (3): shows control and Curve growth of *Salmonella* using Bimedia 205A with Bactrac 4300methods for 24 hrs. coliform Bacteria

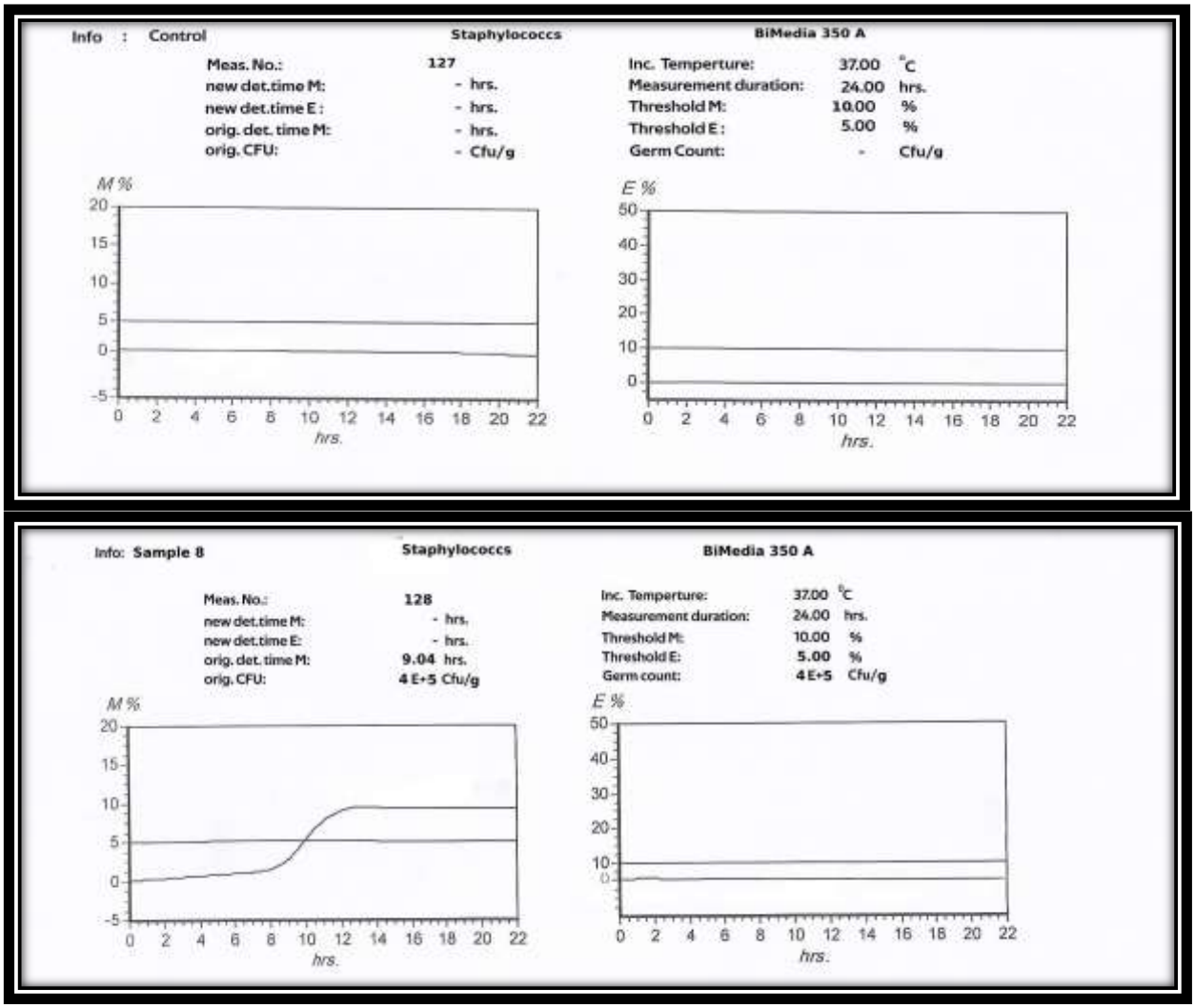


Figure (4): shows control and Curve growth of *Staphylococcus* using Bimedia 350A with Bactrac 4300methods for 24 hrs.

CONCLUSIONS

The study showed that there is a convergence in the results of the two methods used, as the total number of aerobic bacteria (Total plate count), Total Coli From Bacteria, and *Staphylococcus aureus* bacteria are higher than the limits allowed in the Iraqi standard specification for dairy and grafted ice cream No.(702).for the year 1984. While the study samples were clear from *Salmonella* spp.

RECOMENDACTIONS

This study urged the researchers to conduct a number of studies regarding other dairy products, as well as to avoid eating ice cream from unreliable sources.

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