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# Effect of Adding of Bacteriocin Produced from Streptococcus thermophilus Bacteria on the Microbial Content and Some Pathogenic Bacteria in Soft Cheese

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## **Article Informations**

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# ABSTRACT

This study was conducted at the College of Agriculture at Tikrit University for the period from the beginning of May until the end of September 2022. The effect of adding bacteriocin produced from Streptococcus thermophilus bacteria in the manufacture of soft cheese was studied. The cheese was made from pasteurized cow's milk with the addition of (0.5%, 1%, 1.5%, 2% of bacteriocins) and its microbial content (aerobic bacteria, coliform bacteria, yeasts and molds) was studied during the refrigerated storage period (at  $5 \pm 1$ °C). For 28 days. And a study of the effect of bacteriocins on (Staphylococcus aureus, Bacillus cereus, Escherichia coli) as pathogenic bacteria in soft cheese. The results of the statistical analysis of these factors showed that there were significant differences in the total numbers of microorganisms in the soft cheese produced. The addition of bacteriocin had a clear effect on all types of microorganisms under study during the storage period.



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# Introduction

Eliminating the cumulative effect of the use of industrial preservatives on the health of the consumer is a concern for those interested and working in the field of food manufacturing. New food preservation strategies have become important and successful in food processing and preservation. These strategies for the food preservation process aim to eliminate the microorganisms that cause food spoilage, and to preserve the original nutritional value and sensory characteristics of the food. The recent increase in the concept of freshlike foods and minimally processed foods has promoted the search for innovative ways to preserve food by preventing food-borne pathogens and microbial spoilage, thus ensuring food safety, quality, freshness and sensory characteristics. The preservation process generally consists of: Thermal, drying, salting, cold storage and/or using modern preservation techniques such as: canning, pasteurization, and adding chemical preservatives to delay food spoilage and increase the shelf life of produced foodstuffs, due to the latter's negative effects Due to the cumulative impact on the health of the consumer, the trend has been towards the use of bacteriocins Which are peptides manufactured from ribosomes produced by many bacterial strains, as they have been approved as natural compounds due to their decomposition by digestive enzymes in the stomach [1]. The scientist Gratia conducted the first study on bacteriocin in 1925, and it was called Colicin in 1949. It was later called Microcin, and the term bacteriocin was not used until 1953, as the first observation of nisin's activity was in 1971. It was studied by the FDA and was approved as a food additive in 1983 [2].

Bacteriocins have been defined as protein compounds produced by microorganisms to prevent the growth of similar or closely related bacterial strains [3]. The advantage of using bacteriocin as a biopreservative is that it is: nontoxic, easily hydrolyzed by proteolytic enzymes, harmless to gut microbes, and stable over a wide range of pH and temperature [4].

In order to preserve the health of the consumer through the use of vital and completely safe materials, this study aimed to:

1- Taking advantage of microorganisms and their metabolic products and replacing them with artificial preservatives that have a negative impact on the health of the consumer. 2- Study the ability of Streptococcus thermophilus bacteria to produce bacteriocins and study the optimal conditions for production.

3- Using these bacteriocins to prolong the shelf life of some dairy products.

### Materials and methods

This study was conducted at the College of Agriculture at the University of Tikrit for the period from the beginning of May until the end of September 2022. The addition of different proportions of bacteriocins to the shelf life of soft cheese was studied.

After collecting and tabulating the data, it was statistically analyzed according to the experimental system used in the experiment (completely randomized design (CRD) using the Statistical Analysis System (SAS) program[5]. according to the least significant difference at the level of 0.05, and the averages were compared according to the Duncan Multiple Range method. [6].

1- The agricultural media used to conduct the study

(Nutrient Agar, Potato Dextrose Agar (PDA), MacConkey Agar, MRS broth, MRS agar)

2- The bacteria used to complete the study

Streptococcus thermophilus, Staphylococcus aureus, Bacillus cereus, Escherichia coli

3- Preparing the solutions used in the study

a- Peptone water solution (0.1%)

It is prepared by dissolving 1 gm of peptone in 100 ml of distilled water and adding the volume to 1000, then 9 ml of it is placed in tubes that are sterilized in a freezer at a temperature 121°C for 15 minutes [7].

b- Sodium chloride solution (Nacl) 2%

It is prepared by dissolving 20 g of sodium chloride in 100 ml of distilled water, adding the volume to 1000, then placing 225 ml of it in 250 ml glass flasks It was sterilized in an incubator [7].

Physiological Saline Solution

This solution is prepared as stated by [8] by dissolving 8.5 g of sodium chloride in 1000 ml of distilled water, then sterilizing it in an autoclave and keeping it at 4°C When used.

4- Preparing the agricultural media

a- The prepared culture media (Nutrient Agar, Potato Dextrose Agar (PDA), MacConkey Agar, MRS broth, MRS agar) were prepared according to the instructions of their manufacturers and were sterilized with an autoclave 121 Celsius for 15 minutes.

b- Denatured liquid MRS medium:

MRS medium was prepared according to

[9].

# Activation of *Streptococcus thermophilus* bacteria

The bacteria producing bacteriocin were activated at a temperature of 42°C for 24 hours. They were activated in three stages on three consecutive days by taking (1 ml) of their first activation, and it was inoculated in the MRS culture medium, then it was kept at the same temperature and time, after which (1 ml) was taken of this activation, and it was inoculated in MRS medium, and the third activation was also kept at the same temperature and time [10, 11].

## Method of purifying bacteriocin produced from *Streptococcus thermophilus*

- The bacterial strain that produces bacteriocin is grown in the appropriate growth medium with 2% yeast extract and under appropriate production conditions.

- The bacterial cells are killed after the end of the incubation period using a water bath at 70 degrees Celsius for 30 minutes, then cooled to room temperature, then the pH is set to 6.5 and the flask is placed on a rotating shaker at a speed of 200 revolutions/minute for an hour Figs so that the bacteriocins are available Adsorption to killed cells [2].

- Then, centrifuge is used at 5,000 rpm for 15 minutes to separate the bacteriocins adsorbed on the cells and get rid of the growth medium.

- Then the cells are washed with sterile phosphate buffer solution (consisting of 0.479 g NaH2Po4 and 0.177 g Na2Hpo4, the volume is supplemented to a liter with distilled water) at a pH of 6.5 in order to be removed The remaining growth medium was then centrifuged at a speed of 5,000 rpm. minute for 20 minutes.

- The bacteriocins are then separated from the killed cells under acidic conditions by using an acidic salt solution with a concentration of 100 mmol (consisting of NaCl weighing 5.85 grams), then the volume is supplemented to a liter with distilled water and the pH is reduced to 1.5 by a humidifier d phosphoric acid) and then use centrifugation at a speed of 10,000. revolutions per minute for 15 minutes [2, 11].

-This filtrate contains Thermophilin, which is precipitated using saturated ammonium sulfate at a concentration of 80% at a temperature of  $10^{\circ}$ C. This filtrate is then stored at 4°C for 24 hours to allow the protein to completely precipitate [12, 13, 2].

# How to confirm that the resulting filtrate is bacteriocins

To ensure that the filtrate was bacteriocin and not another inhibitor, this filtrate was heated at a temperature of 70°C for 30 minutes, and the test bacteria were grown on the appropriate nutrient media (usually solid nutrient media), and these bacteria were diluted in pipet water Then, 100 micrometers were scanned The bacteria were suspended superficially on the nutrient medium individually (for each bacteria separately). Then, by etching, the inhibitory effectiveness of the filtrate against the test bacteria was tested. The development plates were then incubated at 37 degrees Celsius for 24 hours, and areas of inhibition were observed. This indicates that the resulting filtrate It is a bacteriocin, because bacteriocins are not affected by the temperature specified in this test [14, 15].

### Soft cheese manufacturing:

After receiving the milk, it was pasteurized at 80-85 °C for 30 minutes, after which the milk was divided into 5 parts (5 liters) for each treatment, and the following additives were used:

1- The first section (A) is the control group, which was left without the addition of bacteriocin.

2- The second section (B1) was made with the addition of bacteriocin at a rate of 0.5%

3- The third section (B2) was made with the addition of bacteriocin at a rate of 1%

4- The fourth section (B3) was made with the addition of bacteriocin at a rate of 1.5%

5- The fifth section (B4) was made with the addition of bacteriocin at a rate of 2%

The cheese was manufactured by following the steps mentioned by [16], where the milk was cooled until it reached a temperature of  $(45\pm1)$ Celsius, then bacteriocins were added to their treatments individually, after which the yogurt starter was added (with stirring), then calcium chloride after dissolving it with warm water. The microbial rennet (chymosin enzyme) was added after it was dissolved with distilled water according to the instructions of the producing company, and the pathogenic bacteria were added (in their treatments) with logarithm numbers ranging between (5.3 - 6.2 c.f.u/g) and after half an hour, the results were reached To The desired state of the clot, then this clot was cut into cubes and left for 5 minutes (static), then the whey was drained. The clot was then placed in containers containing holes after wrapping it with pieces of gauze, then pressed (with a weight equivalent to approximately 6-7 kg) to get rid of the largest amount. A quantity of whey, after which the resulting cheese was placed in the refrigerator at a temperature of  $(6\pm1)$  Celsius to study its chemical composition and microbial content after 1, 7, 14, 21, and 28 days of storage.

### Microbiological tests for produced cheese

Estimating the total number of aerobic bacteria. Estimating the total number of colon bacteria. Estimating the total number of yeasts and molds. Estimating the total number of pathogenic bacteria.

### **Results and discussion** Microbial content of cheese Total number of aerobic bacteria

Table (1) shows the effect of adding different percentages of bacteriocins on the total number of aerobic bacteria in soft cheese. It is clear from the results of this study that the logarithm of the bacterial numbers at the third dilution  $(10^3)$  on the first day of storage was 3.65 c.f.u/gm for the cheese treated as control and then transferred It rose to reach 5.25 c.f.u/gm on the twenty-eighth day, while These ranges for all cheese treatments studied during the storage period ranged between 3.30 - 1.71 c.f.u/gm on the first day of storage and 2.40 - 1.89 c.f.u/gm on the eighth day The twentytwentieth period of storage for cheese made with the addition of 0.5 %, 1%, 1.5%, 2% bacteriocin. It is noted from the results that the total number of aerobic bacteria in the cheese of all treatments was less than the cheese of the control treatment.

**Table 1.** Logarithm of the total number of aerobic bacteria in the cheese produced during the storage period (c.f.u/g)

T.	cheese Age (Day)				
Code	1	7	14	21	28
A*	3.65 <sup>Ae</sup>	3.97 <sup>Ad</sup>	4.25 <sup>Ac</sup>	5.07 <sup>Ab</sup>	5.25 <sup>Aa</sup>
B1*	3.30 <sup>Ba</sup>	3.00 <sup>Cb</sup>	$2.06^{\text{Be}}$	2.25 <sup>Bd</sup>	2.40 <sup>Bc</sup>
B2*	3.10 <sup>Cb</sup>	$3.21^{Ba}$	1.90 <sup>Ce</sup>	$2.07^{Bd}$	2.15 <sup>Cc</sup>
B3*	$3.00^{Da}$	$2.03^{\text{Db}}$	1.87 <sup>Cd</sup>	1.88 <sup>Cd</sup>	1.97 <sup>Dc</sup>
B4*	2.97 <sup>Ea</sup>	$2.00^{\text{Db}}$	1.71 <sup>De</sup>	$1.78^{\text{Dd}}$	1.92 <sup>Ec</sup>

A\* Without adding.

(B1\* with the addition of 0.5% bacteriocin). (B2\* With the addition of 1% Bacteriocin). (B3\* With the addition of 1.5% bacteriocin). (B4\* With the addition of 2% Bacteriocin).

The results of the statistical analysis showed that there were significant differences at the 0.05 probability level in the total numbers of aerobic bacteria for some different cheese treatments The numbers of these bacteria increased with the percentage of bacteriocin added, as their numbers in the cheese of the treatment produced with the addition of 2% of bacteriocin were less than the numbers in other cheese treatments produced with concentrations Different types of bacteriocins, and the numbers of aerobic bacteria increased significantly during the storage period in the control cheese, as a result of the doubling of the numbers of live cells or the growth of other types of bacteria The modesty of the microstructure due to the change in the chemical composition of the cheese as the storage period advances.

The results of this study are consistent with what [17] indicated that bacteriocins produced from lactic acid bacteria have the ability to inhibit some types of nearby bacteria that grow And with it in the same environment, when they studied the activity of bacteriocin produced by the bacteria Lactococcus lactis - isolated from some dairy products - Antibiotics to other microorganisms. And the findings of [18] on the effect of the nisinproducing bacterium Lactococcus lactis on the expression of the inlA, plc and hly genes related to the virulence factors of the pathogenic Listeria monocytogenes bacteria Riocin significantly affected the growth, activity, and virulence of the studied pathogenic bacteria.

These results also agreed with the findings of [11] who indicated a decrease in the total numbers of aerobic bacteria in soft cheese when different proportions were added A roll of bacteriocin produced from Lb bacteria. bulgaricus during the 21-day storage period compared to the control treatment. The logarithm of the total numbers on the third day of storage for the cheese of the control treatment and the treatments added with bacteriocins reached 0, 5, 10, 15 ml/kg 4.600, 4.280, 4.240, 4.100 respectively, then These numbers began to increase in the cheese of the control treatment and decrease in the cheese of the bacteriocin addition treatments, reaching on the twenty-first day of the storage period to: 6.130, 3.300, 3.260, 3.200, in the successive order above, which indicates the inhibitory effectiveness of bacteriocin produced by lactic acid bacteria against some species. Other bacteria. Also mentioned by [19] who concluded that the bacteriocin thermophilin 110, produced by the Streptococcus thermophilus bacterium, has an anti-opportunistic pathogenic activity that infects the skin in humans by noting the inhibition zones around the colonies of these bacteria Bacteria when studied with the causes of acne infections.

#### The Total Number of Coliform Bacteria

The results of this study showed that coliform bacteria were unable to grow in all treatments of the soft cheese produced. This is evidence that healthy conditions for manufacturing and production are available and that pasteurization leads to the elimination of this Bacteria and their presence in the milk product is evidence of contamination occurring after pasteurization, and this is what was mentioned by [20] and [11] and the non-appearance of coliforms in soft cheese continued throughout the storage period. This is also due to the effect of the low storage temperature, as well as the effect of starter bacteria, as stated in agreement with It was mentioned by [21] and [22] stated that the bacteria Streptococcus thermophilus and Lactobacillus bulgaricus have the ability to produce substances that inhibit many microorganisms, including colon bacteria. The results of the study were also consistent with what was reported by [23] in that E. coli bacteria were unable to grow in cheese samples to which bacteriocin was added as a preservative. These results were consistent with the findings of [24] regarding the lack of growth of E. coli bacteria after pasteurization of milk used in the manufacture of all cheese treatments It was grown in a laboratory, as was what [25] found, as he stated that coliform bacteria were unable to grow in the cheese of all processed products. From pasteurized milk.

These results were consistent with what [26] and [27] reported, which is that adding lactic acid bacteria or one of their metabolites, such as bacteriocins, when making soft cheese Irrigation leads to inhibiting the growth of coliform bacteria and restricting the growth and activity of other types of microorganisms in cheese. The results of this study were also consistent with what was reported by [11] as it concluded that coliform bacteria were unable to grow in cheese made with the addition of different proportions A roll of bacteriocin produced from lactic acid bacteria.

### **Total Number of Yeasts and Molds**

The results shown in Table (2) showed the effect of adding bacteriocin on the total numbers of yeasts and molds in soft cheese, as no growth of yeasts and molds was observed at the beginning of the period of storage and then began to grow in the second week of storage in the control treatment. The logarithm of their total number in This day is 1.21, reaching 1.35 c.f.u/gm on the twenty-eighth day.

**Table 2.** Logarithm of the total number of yeasts and molds in the cheese produced during the storage period (c.f.u/g)

Τ.	cheese Age (Day)				
Code	1	7	14	21	28
A*	Ad 0.0	$0.0^{\rm Ad}$	1.21 Ac	1.27 <sup>Ab</sup>	1.35 <sup>Aa</sup>
B1*	Aa0.0	0.0 <sup>Aa</sup>	$0.0^{\text{Ba}}$	$0.0^{\text{Ba}}$	$0.0^{\mathrm{Ba}}$
B2*	$0.0^{\rm Aa}$	0.0 <sup>Aa</sup>	$0.0^{Ba}$	$0.0^{\text{Ba}}$	$0.0^{\text{Ba}}$
B3*	0.0 <sup>Aa</sup>	0.0 <sup>Aa</sup>	0.0 <sup>Ba</sup>	0.0 <sup>Ba</sup>	$0.0^{Ba}$
B4*	$0.0^{\mathrm{Aa}}$	0.0 <sup>Aa</sup>	$0.0^{\mathrm{Ba}}$	$0.0^{\text{Ba}}$	$0.0^{\text{Ba}}$

A\* Without adding.

( B1\* with the addition of 0.5% bacteriocin). (B2\* With the addition of 1% Bacteriocin). (B3\* With the addition of 1.5% bacteriocin). (B4\* With the addition of 2% Bacteriocin).

The results of the statistical analysis showed that there were no significant differences in the numbers of yeasts and molds between the cheeses of the different treatments. The use of bacteriocin inhibited the growth of yeasts and molds throughout the period of storage. 28 days, while growth occurred in the second week of this period in the cheese of the control treatment and taking the logarithm of the numbers of these The organisms tend to increase as the storage period progresses.

These results agreed with the findings of [25] that yeasts and molds were not able to grow in the cheese of all treatments until after a week of storage. What [28] concluded that the appearance of yeasts and molds in pasteurized cheese transactions occurred after the seventh day of storage, and then their numbers began to increase until the end of the 21-day storage period, he explained this as a possibility Contamination of cheese after manufacturing. The results of this study are also consistent with what [24] reported that no growth of yeasts and molds appeared during the storage period in soft cheeses manufactured with the addition of bacteriocins. These results were consistent with the findings of [11] that all cheese treatments produced with the addition of bacteriocins were free of yeasts and molds from the first day of storage until the end On the fifteenth day of this period, yeasts and molds began to grow in the cheese of the control treatment, and the logarithm of their numbers reached 1.147, reaching The twenty-first day of storage to 1.270, with its continued inability to grow in cheese treated with the addition of bacteriocin in different proportions and over the duration of storage. The results were also consistent with what was found by [29] who indicated the inhibitory ability of bacteriocins to prevent the growth of many microorganisms,

including yeasts and molds Their study on the biopreservation of cottage cheese.

# Growth and presence of pathogenic bacteria in laboratory-produced soft cheese

The effect of bacteriocin produced by Streptococcus thermophilus on the growth and presence of some types of pathogenic bacteria (positive and negative) for the K dye was studied Ram, as Escherichia coli (negative bacteria), Staphylococcus aureus, and Bacillus cereus (positive bacteria) were selected and grown in samples of soft cheese produced in this The study then studied their numbers and revealed their presence or not due to the inhibitory activity of bacteriocins compared to the control treatment over the length of the storage period of 28 days. The results were estimated in logarithms as in the tables below:

**Table 3.** Logarithm of the numbers of *Escherichia coli* bacteria in the cheese produced during the storage period (c.f.u/g)

Τ.	cheese Age (Day)				
Code	1	7	14	21	28
A*	<sup>Aa</sup> 6.1	5.7 <sup>Aa</sup>	5.2 <sup>Ab</sup>	4.8 Ab	4.2 Ac
B1*	<sup>Aa</sup> 6.0	5.5 Ab	5.1 Ab	4.3  Bc	4.1 cA
B2*	<sup>Aa</sup> 6.0	5.4 <sup>Ab</sup>	4.9 Ac	4.1 <sup>Bd</sup>	3.9 <sup>Ad</sup>
B3*	<sup>Aa</sup> 5.8	5.2 Ab	4.3 <sup>Bc</sup>	$4.0 \ ^{\mathrm{Bc}}$	3.5 <sup>Be</sup>
B4*	<sup>Ba</sup> 5.5	$4.8 ^{\text{Bb}}$	3.6 <sup>Cc</sup>	3.0 <sup>Cd</sup>	2.1 <sup>Ce</sup>

A\* Without adding.

( B1\* with the addition of 0.5% bacteriocin). (B2\* With the addition of 1% Bacteriocin). (B3\* With the addition of 1.5% bacteriocin). (B4\* With the addition of 2% Bacteriocin).

The results of the statistical analysis of the values in Table (3) showed that there are significant differences in the logarithm of the numbers of Escherichia coli bacteria present in the soft cheese produced in this study And the effect of adding bacteriocin in different proportions compared to the control treatment, as it is noted from the table above that the logarithm of the numbers decreases with the progression of the storage period of 28 days. The range of these numbers on the first day of storage ranged between 5.5 for the cheese treated with 2% bacteriocin addition and 6.1 for the cheese treated with the control treatment, and an effect can be seen from the table The ratio of the addition of bacteriocin to the numbers of Escherichia coli bacteria in the cheese samples to which it was added is the logarithm of the numbers of E. coli in the cheese treatment made with the

addition of 0.5% of bacteriocin produced from the bacteria *Streptococcus thermophilus* 6.0, while in the cheese treatment with the addition of 2% of the bacteria The logarithm of the numbers reached 5.5, which indicates the inhibitory activity of bacteriocin against E. coli bacteria, and the increase of this activity with an increase in the concentration of bacteriocin in the medium.

These results agreed with the findings of [30] that E. coli bacteria were not completely eliminated despite the use of bacteriocin as a preservative and the numbers of E. coli were affected This bacteria decreased slightly during the storage period of 28 days. [2] mentioned the observation of a slight decrease in the logarithm of the numbers of E. coli bacteria over the length of the storage period of soft cheese, which was 28 days, and the extent of the effect of adding Lactococcus bacteria and purifying bacteriocin Partially, as it was noted that there were significant between the differences control treatment compared to the addition treatment.

The results of this study also converged with what was indicated by [11] regarding the inhibitory ability of bacteriocin produced by Lb bacteria. bulgaricus against Escherichia coli bacteria by observing the measurement of the diameters of the inhibition zone, which reached (16 mm) at different temperatures for a period of 10 minutes.

**Table 4.** Logarithm of the numbers of *Staphylococcus aureus* bacteria in the cheese produced during the storage

 period (c.f.u/g)

	0,					
Τ.	cheese Age (Day)					
Code	1	7	14	21	28	
A*	6.1 <sup>Aa</sup>	5.9 <sup>Aa</sup>	5.2 <sup>Ab</sup>	3.5 <sup>Ac</sup>	2.1 <sup>Ad</sup>	
B1*	$5.2^{Ba}$	2.5 <sup>Bb</sup>	$0.0^{Bc}$	$0.0^{Bc}$	$0.0^{Bc}$	
B2*	4.3 <sup>Ca</sup>	$0.0^{\text{Cb}}$	$0.0^{\mathrm{Bb}}$	$0.0^{\mathrm{Bb}}$	$0.0^{Bb}$	
B3*	$3.8^{Da}$	$0.0^{Cb}$	$0.0^{\mathrm{Bb}}$	$0.0^{\mathrm{Bb}}$	$0.0^{Bb}$	
B4*	$2.3^{Ea}$	$0.0^{\text{Cb}}$	$0.0^{Bb}$	$0.0^{\mathrm{Bb}}$	$0.0^{Bb}$	

A\* Without adding.

(B1\* with the addition of 0.5% bacteriocin). (B2\* With the addition of 1% Bacteriocin). (B3\* With the addition of 1.5% bacteriocin). (B4\* With the addition of 2% Bacteriocin).

The results of the statistical analysis of the values in Table (4) showed that there were significant differences in the logarithm of the numbers of *Staphylococcus aureus* bacteria present in the soft cheese under study as the storage period increased by 28 days and the effect of adding bacteriocin in different proportions compared to the control treatment, as it is noted from the table above that the logarithm of the numbers decreases with the progression of the period During storage, the range of these numbers on the first day of

storage ranged between 2.3 for the cheese treated with 2% bacteriocin addition and 6.1 for the cheese treated with the control treatment, as can be seen from the cheese data There is a very clear effect of the rate of addition of bacteriocin on the numbers of Staphylococcus aureus bacteria in samples of cheese to which the additive was added. The logarithm of the numbers of these bacteria in processed cheese treated with the addition of 0.5% of bacteriocin produced from Streptococcus thermophilus bacteria was 5.2, while in cheese When adding 2% of bacteriocin, the logarithm of the numbers reached 2.3 on the first day of storage, which indicates the high inhibitory effectiveness of bacteriocin against S. aureus bacteria. It was noted that this activity increased with an increase in the concentration of bacteriocins in the growth medium, and the numbers decreased after the first day until reaching the end of the storage period, when on the twenty-eighth day of this period it reached (zero) for all cheese Treatments with the exception of the control treatment in which the logarithm of the numbers of S. aureus bacteria reached 2.1 This decrease is attributed to a change in the chemical nature of the medium and a natural increase in the numbers of lactic acid bacteria and the secretion of their metabolites into the growth medium, thereby affecting their competitors in the medium.

The results agreed with the study of [31] who found that the diameters of the inhibition zones of the test bacteria (S. aureus) were 15 mm when using bacteriocin produced by BEC Streptococcus thermophilus, and the findings of [30] regarding the decrease in the numbers of S. aureus when using bacteriocin as a biopreservative and no growth of the test bacteria appeared on the twenty-eighth day of storage. These results were also consistent with the results of the study of [2] who concluded that the use of bacteriocins with Lactococcus bacteria in cheese manufacturing led to a log reduction in the numbers of S. aureus bacteria To zero after the first week of the storage period until the end of this period. These results also agreed with what was indicated by [32] and [11] that the use of bacteriocins to inhibit the test bacteria led to the appearance of inhibition zones with diameters not exceeding 2 Less than (14 mm) at different temperatures.

**Table 5.** Logarithm of the numbers of *Bacillus cereus* bacteria in the cheese produced during the storage period (c.f.u/g)

· 0/						
Τ.	cheese Age (Day)					
Code	1	7	14	21	28	
A*	5.8 <sup>Aa</sup>	5.6 <sup>Aa</sup>	5.2 <sup>Ab</sup>	5.2 <sup>Ab</sup>	4.7 <sup>Ac</sup>	
B1*	$5.0^{\text{Ba}}$	2.9 <sup>Bb</sup>	$0.0^{Bc}$	$0.0^{Bc}$	$0.0^{Bc}$	
B2*	4.1 <sup>Ca</sup>	$0.0^{\mathrm{Db}}$	$0.0^{\mathrm{Bb}}$	$0.0^{\mathrm{Bb}}$	$0.0^{\mathrm{Bb}}$	
B3*	4.0 <sup>Ca</sup>	$0.0^{\mathrm{Db}}$	$0.0^{\mathrm{Bb}}$	$0.0^{\mathrm{Bb}}$	$0.0^{\mathrm{Bb}}$	
B4*	$3.5^{Da}$	$0.0^{\text{Db}}$	$0.0^{\mathrm{Bb}}$	$0.0^{\mathrm{Bb}}$	$0.0^{\mathrm{Bb}}$	

A\* Without adding.

(B1\* with the addition of 0.5% bacteriocin). (B2\* With the addition of 1% Bacteriocin). (B3\* With the addition of 1.5% bacteriocin). (B4\* With the addition of 2% Bacteriocin).

The results of the statistical analysis of the values in Table (5) indicate that there are significant differences in the logarithm of the numbers of Bacillus cereus bacteria in the treatments of soft cheese manufactured in H This study advances the storage period of 28 days and the effect of adding different percentages of bacteriocin produced from Streptococcus thermophilus bacteria compared to the control treatment. From the table, the logarithm of the numbers decreases with the progression of the storage period. The range of these numbers on the first day of storage ranged between 3.5 for cheese treated with 2% bacteriocin addition and 5.8 for cheese Between the control treatment, it is also noted from the data that the effect of the bacteriocin addition rate clearly on the numbers of Bacillus cereus bacteria in Treatment of cheese produced with the addition of bacteriocin. The logarithm of the numbers of these bacteria in the treatment of cheese produced with the addition of 0.5% of bacteriocin produced was Streptococcus thermophilus bacteria 5.0, while in cheese treated with the addition of 2% bacteriocin, the logarithm of the numbers reached 3.5 on the first day of storage. This is evidence of the inhibitory ability of bacteriocin. Against Bacillus cereus bacteria, it is noted that the inhibitory activity of bacteriocin increased with an increase in its concentration in the growth medium, then the numbers decreased after the first day until reaching the end of the storage period, when it reached the twenty-eighth day of this period To (zero) to neutralize all transactions except the control transaction that reached the logarithm of numbers B. cereus bacteria contains 4.7.

The results of this study agreed with what [33] found that bacteriocin has a high ability to

inhibit the growth of B. cereus bacteria in laboratory-made soft cheese. The results were also close to the same A finding by [2] of the effect of bacteriocin on the numbers of *B. cereus* bacteria in cheese The laboratory-produced fresh meat and the logarithm of these numbers decrease from the first day of the storage period until no growth of the test bacteria is observed on the seventh day until the end of the storage period. These results also agreed with the diameters of the inhibition zones of the test bacteria (*B. cereus*) when [11] used bacteriocin as an inhibitor of this bacteria, as the diameters of the inhibition zones reached a range between (18-20 mm) and at temperatures ranging between 40-100 degrees Celsius for 10 minutes.

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