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EFFECT OF ADDITION RAW BACTERIOCIN PRODUCED BY *Lactobacillus delbrueckii* Sub-Sp. *bulgaricus* ON SOFT CHEESE

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ABSTRACT

The effect of adding raw bacteriocin produced by *Lactobacillus bulgaricus* to cheese curd at an amount of (5 and 10 and 15) mL/kg cheese as a biological preservative to prolong the shelf life of soft cheese, in addition to the control treatment, knowing that each 1 mL of bacteriocin filter contains 15 units/ mL of bacteriocin. The results of the physicochemical, microbial and sensory tests for cheese stored at refrigerator temperature for a period (zero) to (21) d of adding bacteriocin showed the superiority of the treatment of cheese added to 15 mL/kg cheese of bacteriocin over the rest of the other treatments during the storage period, where the acidity development decreased by 0.363 compared to Cheese with the control treatment, which was at a rate of 0.547, as well as a significant decrease in the number of microorganisms compared with the cheese treated with the control treatment, as well as a clear improvement in the sensory characteristics of soft cheese, which makes it qualified to be a biological preservative for the absence of negative effects on the properties of the obtained product.

Keywords: Bacteriocins, *Lactobacillus bulgaricus*, shelf life, soft cheese.

تأثير إضافة البكتريوسين الخام المنتج من بكتريا *Lactobacillus delbrueckii* Sub-Sp. *bulgaricus* على الجبن الطري

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الخلاصة

دُرس تأثير إضافة البكتريوسين الخام المنتج من بكتريا *Lactobacillus bulgaricus* الى خثرة الجبن بمقدار 5 و10 و15 مل/كغم جبن كمادة حافظة حيوية لاطالة العمر الخزني للجبن الطري، فضلاً عن معاملة السيطرة علماً ان كل 1 مل من راشح البكتريوسين يحوي على 15 وحدة/مل من البكتريوسين، واظهرت نتائج الفحوصات الفيزوكيميائية والميكروبية والحسية للجبن المخزون بدرجة حرارة الثلاجة وللفترة من صفر الى 21 يوم من إضافة البكتريوسين تفوق معاملة الجبن المضاف اليه 15 مل/كغم جبن من البكتريوسين على بقية المعاملات الاخرى خلال مدة التخزين، حيث انخفض تطور الحموضة بنسبة 0.363 مقارنةً مع الجبن معاملة السيطرة التي كانت بنسبة 0.547 وكذلك انخفاض في اعداد الاحياء المجهرية بشكل ملحوظ مقارنةً مع معاملة السيطرة، فضلاً عن ظهور تحسن واضح في الصفات الحسية للجبن الطري، مما يجعله مؤهلاً لأن يكون كمادة حافظة حيوية لعدم وجود تأثرات سلبية على خصائص المنتج المستحصل عليه.

الكلمات المفتاحية: البكتريوسين، *Lactobacillus bulgaricus*، اطالة العمر الخزني، الجبن طري.

INTRODUCTION

The advantage of lactic acid bacteria is that they are important in food and therapeutic processing and have multiple characteristics that they grow in aerobic and anaerobic conditions, their rapid growth, non-producing toxins, most of them are non-pathogenic, and they resist low pH, and their production of flavoring materials in food as well as their ability to preserve food because of their products The metabolites resulting from fermentation, such as bacteriocins, have been used as probiotics, which are single or combined cultures of microorganisms that have beneficial effects on health, and are important in treating many diseases that affect humans and animals **(Seddik *et al.*, 2017)**, Interest in biopreservation has increased since the 1980s as a new method for controlling pathogenic or spoiling bacteria in food and dairy products. Interest in using lactic acid bacteriocins in preservation, as natural biopreservatives **(Daba *et al.*, 2021)** has increased significantly. The bacteriocins are stable towards temperature and pH. The bacteriocins have low molecular weights, and the bacteriocins maintain the balance of the natural flora **(Hamel *et al.*, 2020; Zou *et al.*, 2018)**. It possesses *Lactobacillus delbrueckii sub-sp. bulgaricus* has anti-bacterial, immune-stimulating and anti-mutagenic properties, and an important feature is its ability to produce bacteriocins, as it creates peptides in the extracellular ribosomes, where it works to form stomata on the cytoplasmic membrane, which results in the depolarization of the membrane, which causes the exudation of essential cellular substances such as ions and the depletion of energy ATP and potassium which leads to cell destruction and thus death **(Boyanova *et al.*, 2017)** and can inhibit disease-causing bacteria such as *Listera monocytogenes* **(Scatassa *et al.*, 2018)**. There are at least three ways in which bacteriocins can be incorporated into a food part to improve its safety: using a purified or semi-purified bacteriocin preparation as an ingredient in the food, by incorporating an ingredient that has been previously fermented with bacteria and producing a strain, or using a culture to replace all or part of the food. A starter in fermented foods to produce bacteriocin on site. Bacteriocin can also be used to improve food quality and organoleptic properties, increase the rate of protein decomposition or prevent the appearance of gas pits in cheeses. Bacteriocin can also be used for biological packaging, a process that protects food from external contamination, which improves food safety and shelf life **(Mezher & Al-Bayyar, 2018; Singh, 2018)**. That bacteriocins are natural and safe products (GRAS), and can be used as a food preservative, and that nisin is the only bacteriocin widely used in food preservation, so research must continue to officially approve the use of new bacteriocins in food preservation **(Oconnor *et al.*, 2020)**, So, The research aim was use of bacteriocin produced by *Lactobacillus bulgaricus* to extend the shelf life of soft cheese and the extent of its impact on the chemical, microbial and sensory properties of soft cheese.

MATERIAL AND METHODS

Activation of bacterial isolation

The bacterial isolate was activated by taking 0.2 g of the initiator, inoculated in 20 mL of MRS liquid medium, *Lactobacillus bulgaricus* was incubated at a temperature of 37°C for 24 d, as it became active during three consecutive d, by taking 1 mL of the first activation, it was inoculated in MRS medium, kept at temperature and time as mentioned above, and on the third day was taken from the second insemination, and she was inoculated in MRS medium and kept at temperature and time above.

How to prepare the filter

The method of **Kim *et al*. (2019)** was used to prepare bacteriocin filtrate-use the farm centrifuge at 4000 rpm to remove bacterial cells for 20 min at 4°C.

- Adjust the initial pH of the filter to 6.5 using NaOH; to exclude the effect of organic acids in the inhibition.
- The bacteriocin filtrate was filtered through 0.2 µm porosity cellulose acetate membranes; in order to examine the inhibitory activity of the selected bacterial isolates.

Static Fermentation Method

Use a temperature of 37°C for 24 h in aerobic conditions, as 2 mL of bacteriocin-producing cells were grown under aerobic conditions in 200 mL of MRS medium with pH 5.6, and at a temperature of 37°C for 24 h.

Cheese manufacture and addition of bacteriocin produced from *Lactobacillus bulgaricus*

The soft cheese was manufactured according to the method **Lim *et al.* (2020)** with some modifications, where the pasteurized full-fat milk was purchased from the local markets. The milk was divided into four sections, each 5 L separately. The milk was heated to 37°C and the usual rennet was added in the Iraqi markets 0.02% and it was dissolved in water, then salt (NaCl) was added at a rate of 1% and it was cut to drain the most amount of whey, then the whey was drained, then bacteriocin produced from *Lactobacillus bulgaricus* was added at an amount of 5, 10 and 15 mL per kilo of cheese And each separately, knowing that 1 mL of the bacteriocin filtrate contains 15 units/mL of bacteriocin. Then the curd was cut and left for 5 min, then the curd was filled in molds and the cheese was kept in the refrigerator at a temperature of 4°C until an unwanted smell appears. Or the appearance of color or growth of microorganisms,

Sensory, chemical and microbial examinations

Chemical tests for cheese

pH measurement

Weigh 10 g of cheese and mix it well in a ceramic slurry with 10 mL of distilled water, and the pH value was estimated with a pH meter.

Acidity

Weigh 3 g of cheese and mix it well with a ceramic mortar with 10 mL of distilled water and add a few drops of phenolphthalein reagent, then wipe it with 0.1 NaOH solution and the acidity was estimated according to the following equation.

$$\text{Total acidity (TA) (\%)} = \frac{\text{volume of NaOH} \times 0.009 \times 0.1}{\text{weight of sample}} \times 100$$

Moisture percentage

The percentage of moisture in soft cheese was estimated using clean and sterilized drying eyelids and weighed while empty. Then 3 g of cheese was placed and placed in the oven at a temperature of 105°C for 3 h, then weighed after cooling until the weight was stable. The percentage of moisture was estimated through The following equation.

$$\text{Moisture (\%)} = \frac{\text{weight of the sample with the lid before drying} - \text{weight of the sample with the lid after drying}}{\text{weight of sample}} \times 100$$

protein ratio

The percentage of protein in soft cheese in this study was estimated by the method known as (Macro Kjeldahl).

Fat

The percentage of fat in this study was estimated by the Gerber method.

Microbiological examinations

Sampling preparation

The method of sampling and microbial qualitative of cheese was study by taking 11 g of cheese sample from four parts of the outer circumference and distributed at equal distances and taking part of the middle where these parts represent the outer total of cheese as much as possible and use a sterilized knife for this purpose. 99 mL of distilled water containing (0.1% Peptone) in order to maintain the vitality of the bacteria with the addition of 2% sodium citrate to increase the disintegration of the sample. The sample was mixed well through the mixture to complete the homogenization process, then the required dilutions and preparations for the various studies were made **(Zaky & Mahmoud, 2019)**.

Estimate the total number of bacteria

The examination of the total number of bacteria after manufacturing and the length of storage period for each of the treatments was carried out, as the method of pouring in plates mentioned in **APHA (1978)** was followed using a solid nutrient agar medium. The dishes were incubated at 35°C for 48 h.

Estimated total number of coliform bacteria

The examination of the total number of coliform bacteria after manufacturing and the length of storage period for each of the treatments was carried out. The dish pouring method mentioned in **APHA (1978)** was followed using MacConkey agar medium, and the dishes were incubated at a temperature of 37°C for 24 h.

Estimation of the total number of yeasts and molds

An examination of the total number of yeasts and molds after manufacturing and the length of storage period for each of the treatments was carried out. The dish pouring method mentioned in **APHA (1978)** was followed using PDA medium, and the dishes were incubated at of 25°C for 5 d.

Sensory evaluation of cheese

Sensory evaluation of cheese samples was carried out by 5 assessors and was carried out according to sensory evaluation (form 1) and included characteristics, namely taste, texture, appearance and total 20 before conducting chemical and microbial analyzes on them to ensure the validity of the samples for human consumption by presenting cubed pieces of weight 15-20 g of cheese for each resident **(Zaky & Mahmoud, 2019)**.

Form (1): Sensory evaluation of soft cheese

Attributes to be evaluated				nature of the transaction
Appearance (**20** Degree)	Taste (**45** Degree)	Texture (**35** Degree)	the total (**100** Degree)	

Statistical analysis

All laboratory experiments were carried out according to a fully randomized design (Two-way ANOVA in CRD). Means were compared using the LSD test **(Al-Rawi,1980)**. The analysis was done using Excel and Genstat V. 12.1 software **VSN International,(2012)**

RESULTS AND DISCUSSION

Chemical tests for soft cheese

pH

It is noticed from (Table 1) that there was a decrease in the pH value during the storage period in all treatments until the end of the lifespan. From storage, the pH value of the samples added to which bacteriocin produced from *Lactobacillus bulgaricus* was close to that of the cheese samples of the control treatment without addition, except that during cold storage, a decrease in the pH value of the cheese samples was observed from the control treatment compared to the cheese added with bacteriocins. Produced from Lactobacillus bulgaricus more clearly. The pH values in this study ranged in the first day, for all treatments it was 6.4, and these values decreased with the progression of the storage period until it reached 4.1 on the twenty-first day for cheese treated as control.

The results agree with **Dina *et al.* (2013)**, where bacteriocin added to soft cheese had a higher pH value until the end of the storage life. These results agreed with **Felicio *et al.*, (2015)**, where after adding bacteriocin nisin, the pH of cheese decreased due to the increase in storage period.

Table (1): The pH value of soft cheese during the storage period.

Average storage life	Addition rate of bacteriocin (mL/kg) cheese^{-1}				Age (d)
	0	5	10	15	
Zero	6.367	6.367	6.400	6.400	6.383
3	6.200	6.267	6.300	6.400	6.292
6	6.033	6.100	6.100	6.167	6.100
9	5.667	5.900	5.867	6.100	5.883
12	5.467	5.833	5.833	6.033	5.792
15	5.033	5.633	5.733	5.900	5.575
18	4.767	5.367	5.600	5.767	5.375
21	4.100	5.300	5.467	5.700	5.142
0.038	0.076				LSD
	5.454	5.846	5.913	6.058	Average addition
	0.027				LSD

Total acidity

The results (Table 2) shows reached in this study in estimating the total acidity percentage for all treatments of soft cheese during the storage period, and it showed the opposite character of the pH. The acidity percentage of all treatments on the first day of storage ranged 0.16%, then this percentage increased to 0.55% for the treated cheese. The control after the twenty-first day of the storage period, where there are significant differences at the probability level of P≥0.05 between the cheese treated with the control and the cheese added with bacteriocin produced *by Lactobacillus bulgaricus*.

The results agreed with **Zaky & Mahmoud (2019)** and found that the addition of bacteriocin led to a decrease in acidity and an increase in pH during the storage period compared to cheese not treated with bacteriocin and a high inhibitory effect on the total number of bacteria from cheese treatments to which bacteriocins were not added, pasteurization and good cooling and this is what he reached **(Choi *et al.*, 2016)**.

Table (2): The total acidity value of soft cheese during the storage period.

Average storage life	Addition rate of bacteriocin (mL/kg) cheese^{-1}				Age (d)
	15	10	5	0	
0.160	0.160	0.160	0.160	0.160	Zero
0.180	0.167	0.170	0.177	0.207	3
0.223	0.207	0.227	0.227	0.233	6
0.263	0.217	0.260	0.270	0.303	9
0.312	0.260	0.307	0.327	0.353	12
0.365	0.313	0.327	0.380	0.440	15
0.403	0.340	0.360	0.417	0.493	18
0.438	0.363	0.390	0.453	0.547	21
0.028	0.055				LSD
	0.253	0.275	0.301	0.342	Average addition
	0.020				LSD

Moisture percentage

The results shown in (Table 3) indicate that the moisture content in all cheese samples was close due to the same manufacturing method, but there is a slight difference between the control-treated cheese and with the bacteriocin-treated cheese, where there is a slight increase in the control-treated cheese, and its percentage at the end of the storage period is 21 d. On 56.933, while cheese added to it 15 mL of bacteriocin had the lowest moisture content of 56,837. The difference may be due to the heterogeneity of the pressing process for the different treatments**.** Yeasts and molds in control-treated cheese cause the release of proteolytic enzymes. The results agree with what was found by **Zaky & Mahmoud (2019)**.

Table (3): The percentage of moisture in soft cheese during the storage period.

Average storage life	Addition rate of bacteriocin (mL/kg) cheese^{-1}				Age (d)
	15	10	5	0	
58.525	58.503	58.513	58.527	58.557	Zero
58.421	58.397	58.407	58.427	58.453	3
58.136	58.087	58.107	58.133	58.217	6
57.831	57.783	57.803	57.843	57.893	9
57.678	57.637	57.657	57.687	57.733	12
57.487	57.447	57.467	57.487	57.547	15
57.232	57.180	57.207	57.237	57.303	18
56.883	56.837	56.867	56.893	56.933	21
0.007	0.015				LSD
	57.734	57.753	57.779	57.830	Average addition
	0.005				LSD

Protein ratio

It is noted from the results shown in (Table 4) that the percentage of protein in the different cheese samples was very close, due to the use of the same raw material and the same manufacturing method, which is close to the protein percentages in the samples of control cheese and cheese to which bacteriocins were added, and this may be the reason for the high percentage of protein in the samples The length of the storage period at the temperature of the refrigerator itself, and also that the humidity ratios were close in all samples, which leads to the convergence of the protein ratios in the comparison samples with cheese samples to which bacteriocin was added, mentioned that the closeness in the percentage of moisture for several samples of cheese made from the same raw material leads to convergence in the ratios of fat and protein in the same samples

Table (4): The protein content of soft cheese during the storage period.

Average storage life	Addition rate of bacteriocin (mL/kg) cheese^{-1}				Age (d)
	15	10	5	0	
20.632	20.630	20.630	20.630	20.637	Zero
20.660	20.690	20.670	20.650	20.630	3
20.860	20.877	20.863	20.857	20.843	6
21.199	21.223	21.217	21.183	21.173	9
21.483	21.510	21.483	21.467	21.473	12
21.559	21.587	21.563	21.557	21.530	15
21.846	21.863	21.850	21.840	21.830	18
22.167	22.180	22.170	22.160	22.157	21
0.004	0.007				LSD
	21.320	21.306	21.293	21.284	Average addition
	0.003				LSD

Fat percentage

It is noted from (Table 5) that the percentage of fat in all samples was close, as the addition of bacteriocins produced from *Lactobacillus bulgaricus* did not affect the percentage of fat in soft cheese negatively or positively. The convergence in moisture percentages in different cheese samples leads to convergence of fat percentages in the same samples.

Table (5): The fat percentage of soft cheese during the storage period.

Average storage life	Addition rate of bacteriocin (mL/kg))cheese^{-1}				Age (d)
	15	10	5	0	
17.478	17.480	17.480	17.480	17.473	Zero
17.523	17.523	17.523	17.523	17.523	3
17.770	17.790	17.773	17.763	17.753	6
17.958	17.980	17.963	17.950	17.937	9
18.363	18.383	18.373	18.357	18.337	12
18.673	18.683	18.680	18.677	18.653	15
18.863	18.873	18.870	18.860	18.850	18
19.383	19.667	19.467	19.300	19.100	21
0.012	0.025				LSD
	18.298	18.266	18.239	18.203	average addition
	0.009				LSD

Microbiological examinations

Estimate the total number of bacteria

It is noted from (Table 6) the total number of bacteria in the manufacture of cheese treated with bacteriocins and untreated. Significant differences are noted between the control-treated cheese and cheese added to it with bacteriocin produced by *Lactobacillus bulgaricus*. It is noted that there is no number of bacteria at the zero age, which is an indication of the health conditions during manufacturing and there is an increase In the number of bacteria during the storage period for cheese that was not treated with bacteriocins, more than cheeses to which bacteriocins were added, and an increase in the microbial number begins after the age of three days, knowing that the cheese was kept in cold storage at a temperature of 4°C, but it did not prevent an increase in the number of bacteria Cheese that cold storage of soft cheese does not prevent the continuous and clear gradual increase in microbial numbers and the slow growth of bacterial preparation in cheese samples to which bacteriocins produced from Lactobacillus are added. bulgaricus compared to the numbers recorded in cheese samples without treatment to the positive effect of bacteriocins in the cheese-contaminated neighborhoods. The processed cheese that contains 15 mL of bacteriocin produced from *Lactobacillus bulgaricus* has the ability to reduce the total numbers of bacteria more than cheeses that contain 5 and 10 mL of bacteriocin. This indicates the positive effect of bacteriocin in reducing the total numbers of bacteria contaminating soft cheese.

The results are consistent with **Mirdamadi & Ghazvini (2015)** in terms of the decrease in the number of bacteria in white cheese containing bacteriocins until 14 d of manufacture. The results are consistent with **Malas (2016)** the logarithm decrease in the number of Staphylococcus aureus in cheese after adding bacteriocin and up to 21 d of cold storage, and it appears that the number of bacteria increased after 21 d . The reason may be the growth of microorganisms that bacteriocin was not able to inhibit because of the specialization of bacteriocin towards some types of bacteria Microorganisms and not with all of them.

Table (6): Log of the total microbial number of soft cheese treated and untreated with bacteriocin produced from Lactobacillus bulgaricus in periods of cold storage.

Average storage life	**Addition rate of bacteriocin (mL /kg) cheese^{-1}**				**Age (d)**
	15	**10**	**5**	**0**	
0.000	0.000	0.000	0.000	0.000	Zero
4.320	4.160	4.240	4.280	4.600	3
4.330	4.070	4.150	4.210	4.910	6
3.580	3.070	3.070	3.130	5.060	9
3.580	2.840	2.950	3.030	5.490	12
3.620	2.770	2.890	2.940	5.880	15
3.600	2.700	2.840	2.900	5.960	18
3.970	3.200	3.260	3.300	6.130	21
0.011	0.022				LSD
	2.850	2.920	2.980	4.750	average addition
	0.008				LSD

Estimated total number of coliform bacteria

No growth of coliform bacteria was observed in the soft cheese industry, and the cheese samples were free of coliform bacteria. The absence of coliform bacteria in cheese indicates the availability of appropriate health conditions when manufacturing. The absence of coliforms is routinely considered as an indicator of the health quality of food products and their presence indicates unhealthy methods. For production and that pasteurization kills colon bacteria and its presence in the product will be the result of contamination after pasteurization The results agree with **Bolivar *et al.* (2020)** that adding lactic acid bacteria or their metabolites such as bacteriocins to cheese had a negative effect on the presence of coliform bacteria in soft cheese and the results are consistent with **Dina *et al.* (2013)**, failure to detect coliform bacteria in all cheese samples during cold storage until the end of the shelf life

Estimation of the total number of yeasts and molds

It is noted from (Table 7) that all cheese samples containing bacteriocins were completely free of yeasts and molds until the end of the storage period, while the presence of yeasts and molds appeared in the tests that took place 15 d after production for cheese treated as a control and their numbers increased with the progression of the storage period, which indicates the effect positive for bacteriocin produced from *Lactobacillus bulgaricus* because yeasts and molds did not grow in cheeses to which bacteriocin was added. These results are in agreement with **Dina *et al.* (2013)** where yeasts and molds did not grow during the storage period of soft cheese with bacteriocin added. when they found that yeasts and molds began to appear in the second week of storage in soft cheese treated with bacteriocins, meaning that bacteriocins have an inhibitory effect on the growth of molds and yeasts.

Table (7): Logarithm of the total number of yeasts and molds for soft cheese treated and untreated with bacteriocin produced from *Lactobacillus bulgaricus* in periods of cold storage

Average storage life	**Addition rate of bacteriocin (mL/kg) cheese^{-1}**				**Age (d)**
	15	**10**	**5**	**0**	
0.000	0.000	0.000	0.000	0.000	ZERO
0.000	0.000	0.000	0.000	0.000	3
0.000	0.000	0.000	0.000	0.000	6
0.000	0.000	0.000	0.000	0.000	9
0.000	0.000	0.000	0.000	0.000	12
0.287	0.000	0.000	0.000	1.147	15
0.304	0.000	0.000	0.000	1.217	18
0.318	0.000	0.000	0.000	1.270	21
0.524	1.049				LSD
	0.000	0.000	0.000	0.454	Average addition
	0.371				LSD

Sensory evaluation of cheese

Appearance evaluation

It is noted from (Table 8) that the grades given by the assessors for the appearance characteristic of all samples were in general the full degree until the second week of storage with some decrease in the degree for the control cheese samples until the 21 d of storage, which indicates the efficiency of the manufacturing process as well as a decrease in the degree of appearance in the second week of the control-treated cheese samples due to some moldy growths, while the samples added to the bacteriocins produced from *Lactobacillus bulgaricus* retained high levels of the appearance characteristic. Appearance of cheese the samples containing bacteriocin produced by *Lactobacillus bulgaricus* outperformed the best results in terms of appearance, as the samples retained to a large extent their good qualities.

Table (8): The sensory evaluation of soft cheese for appearance in cold storage periods.

Average storage life	Addition rate of bacteriocin (mL/kg) cheese^{-1}				Age (d)
	15	10	5	0	
19.83	20.00	20.00	20.00	19.67	Zero
19.75	20.00	20.00	19.67	19.33	3
19.92	20.00	20.00	20.00	19.00	6
19.00	19.00	19.00	19.00	19.00	9
18.75	19.00	19.00	19.00	18.00	12
18.25	19.00	19.00	18.00	17.00	15
18.00	19.00	19.00	18.00	16.00	18
16.25	17.00	17.00	17.00	14.00	21
0.19	0.37				LSD
	19.08	19.04	18.79	17.96	Average addition
	0.13				LSD

Flavor rating

The characteristic of flavor and taste depends as a basic indicator of the quality of the product and the efficiency of the manufacturing process. The results shown in (Table 9) indicate that the scores awarded by the assessors to the flavor trait were high and equal for all samples, but the significant differences at the probability level of $P \geq 0.05$ in the scores given to the flavor trait appeared with The period of cold storage progressed, and it was in favor of the samples added to bacteriocin produced by *Lactobacillus bulgaricus*. These differences increased in the third week of cold storage, due to the deterioration of the flavor characteristics of the samples of the comparison cheese, so bitterness with a rancid acid taste appeared in these samples. As for the samples added to the bacteriocin, they were given high scores. The variance between the comparison cheese samples and the other samples is due to the variance in the total microbial number between these samples. These results are consistent with what was mentioned by **Khider & Elbanna (2017)** that the comparison cheese had an undesirable flavor compared to cheese with added bacteriocin and that bacteriocin had a clear and positive effect on the product in terms of sensory characteristics.

Table (9): The results of sensory evaluation of soft cheese for its flavor in periods of cold storage.

Average storage life	Addition rate of bacteriocin (mL/kg) cheese^{-1}				Age (d)
	15	10	5	0	
43.42	45.00	43.67	43.00	42.00	Zero
42.42	43.67	43.33	42.67	40.00	3
41.33	42.67	41.67	41.67	39.33	6
35.83	37.33	36.33	35.33	34.33	9
34.42	35.33	34.33	34.33	33.67	12
31.75	34.00	33.00	32.00	28.00	15
28.83	33.00	31.00	30.00	21.33	18
23.42	27.67	26.00	25.00	15.00	21
0.50	1.00				LSD
	37.33	36.17	35.50	31.71	Average addition
	0.35				LSD

Body assessment

It is noted from (Table 10) that the grades given by the assessors for texture were varying and close to the second week, which indicates the efficiency of the manufacturing process and the absence of any chemical changes that lead to an undesirable texture. All samples enjoyed an acceptable normal texture and after a week of cold storage The samples with bacteriocins added retained their full degree for this trait, while the cheese samples of the control treatment decreased their degree compared with the scores given to the samples added with bacteriocins.

Table (10): The sensory evaluation of soft cheese for consistency in periods of cold storage.

Average storage life	**Addition rate of bacteriocin (mL/kg) cheese^{-1}**				**Age (d)**
	15	**10**	**5**	**0**	
35.00	35.00	35.00	35.00	35.00	Zero
35.00	35.00	35.00	35.00	35.00	3
34.42	34.67	34.67	34.67	33.67	6
33.75	34.00	34.00	34.00	33.00	9
32.17	33.00	33.00	32.00	30.67	12
31.75	33.00	32.00	32.00	30.00	15
29.50	32.00	31.00	31.00	24.00	18
24.42	26.67	26.00	25.00	20.00	21
0.25	0.50				LSD
	32.92	32.58	32.33	30.17	Average addition
	0.18				LSD

And from the sensory evaluation, as shown in (Table 11), which shows the total sensory evaluation of the samples of the comparative cheese and cheese with bacteriocin added, which shows the superiority of the treatment of cheese with bacteriocin by 15 mL over the rest of the treatments, meaning there are significant differences between the treatments added to it. Bacteriocin with the comparison cheese, which shows that it is possible to increase the shelf life of soft cheese after 21 d of refrigerated storage, while the control cheese samples deteriorated during the storage period. To the consumer as indicated in the results of microbial tests that the combination of bacteriocins and heat and non-thermal treatments opens innovative possibilities for application in dairy products and a promising progress for microbiological safety and preservation of sensory properties in dairy products.

Table (11): The total sum of sensory evaluation results for soft cheese during periods of cold storage.

Average storage life	**Addition rate of bacteriocin (mL /kg) cheese^{-1}**				**Age (d)**
	15	**10**	**5**	**0**	
98.33	100.00	98.67	97.67	97.00	Zero
97.42	98.67	98.33	97.67	95.00	3
95.75	97.33	96.33	96.33	93.00	6
88.58	90.33	89.33	88.33	86.33	9
85.00	87.33	86.33	85.33	81.00	12
81.75	86.00	84.00	82.00	75.00	15
76.33	84.00	81.00	79.00	61.33	18
64.08	71.33	69.00	67.00	49.00	21
0.51	1.03				LSD
	89.38	87.88	86.67	79.71	average addition
	0.36				LSD

CONCLUSIONS

The addition of bacteriocin produced by *Lactobacillus bulgaricus* to soft cheese lead to an increase in the shelf life and improvement in the chemical, microbial and sensory properties of soft cheese.

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