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USE OF BACTERIOCINS PRODUCED BY SOME LAB AS A NATURAL PRESERVATIVE IN YOGHURT

BY

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ABSTRACT

The effect of bacteriocins produced by *Lactococcus lactis* subsp. *Lactis* ATCC 11454 (T1); *Lactobacillus acidophilus* JCM 1229 (T2); *Lactococcus lactis* subsp. *lactis* ATCC 11454 & *Lactobacillus acidophilus* JCM 1229 (T3) and bacteriocin extracts from *Lactococcus lactis* subsp. *lactis* isolate (T4); *Lactobacillus acidophilus* isolate (T5); *Lactococcus lactis* subsp. *lactis* & *Lactobacillus acidophilus* isolates (T6) as a natural preservative in yoghurt compared with control yoghurt (C1) and yoghurt with nisin (C2) were evaluated. Use of nisin and bacteriocins produced or extracted with a level of ~100 AU/ml. Yoghurt samples were assessed for coagulation time, chemical analysis, rheological properties, microbiological quality, bacteriocin activity, sensory evaluation and shelf-life during storage for up to 28 days at ~ 5°C. Yoghurt treated with nisin or bacteriocins produced or extracted by some LAB increased coagulation time compared with control yoghurt. Existence of bacteriocins retarded growth of lactic acid bacteria, thereby delaying acid production. Progressive increases in acid production during storage were observed in control yoghurt compared with bacteriocins-treated yoghurt. The rheological properties, sensory evaluation and shelf-life of yoghurt were improved, when nisin and bacteriocins were used. The quality and shelf-life of yoghurt containing extracted bacteriocins or nisin were still acceptable after 28 days of storage (T4, C2, T6 and T5 respectively) followed, after 21 days with yoghurt containing bacteriocin producing bacteria (T1, T3 and T2 consequently) compared to 14 days for control yoghurt. It was concluded that the addition of bacteriocins extracted or nisin (~100 AU/ml) to milk could be produce yoghurt with good organoleptic properties and prolonged the shelf-life.

Key words: bacteriocins, nisin, yoghurt, preservatives.

INTRODUCTION

Increasing consumption of yoghurt in tropical and subtropical countries emphasis the need for a preservative method to meet the microbiological safety and quality parameters of the finished product. Due to the mishandling conditions during storage, distribution from the suppliers to the retail chain and wide temperature fluctuations of the refrigeration system during handling, the shelf life of yoghurt can be affected.

The beneficial role of lactic acid bacteria (LAB) and their safety in food fermentation have been well documented. First and formest, by their metabolic activities the shelf-life and safety of fermented food products are increased. In

addition, the aroma, texture and flavour may be improved (Du Toit *et al.*, 2000). Also, lactic acid bacteria produce many different inhibitory substances that prolong the time scale of preservation of the fermented products. The preservative action of LAB in foods results from the formation of metabolites' with antimicrobial activity, e.g. bacteriocins or bactericidal proteins during lactic fermentations, which make them useful in food biopreservation (Oyetayo *et al.*, 2003).

The bacteriocins generally recognized as safe (GRAS), have arisen a great deal of attention as a novel approach to control pathogens in foodstuffs and spoilage organisms (Klaenhammer, 1993). Nisin is the only bacteriocin, has no adverse side effects when ingested and so the U.S. Food and Drug Administration in 1988 designated nisin with GRAS and the World Health Organization (WHO) has approved its use as a food additive (Federal Register, 1988).

Bacteriocin producer strains were not inhibited by its own culture supernatant fluid, which indicated the presence of an immunity mechanism. It is well known that most bacteriocin-producing LAB also produces an immunity protein that protects the cell from self destruction (Klaenhammer, 1993).

So, the present study was carried out to evaluate the use of bacteriocins as a natural preservative in yoghurt to increase its shelf-life.

MATERIALS AND METHODS

-Materials

- Fresh mixed milk (cows and buffaloes, 1:1) were obtained from the herds of Faculty of Agriculture, Moshtoher, Benha University.
- Bacteriocin *extracts* were obtained from *Lactococcus lactis* subsp. *lactis* No.8 (*Lc. lactis* No. 8) and *Lactobacillus acidophilus* No.87 (*Lb. acidophilus* No.87) isolated from raw milk samples (El-Alfy *et al.*, 2007).
- Nisin was obtained as Nisaplin product at the concentration of 10^6 IU/g from Aplin & Barrett Ltd. Trowbridge, Wilts, UK.

- Cultures:

Yoghurt starter consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lb. bulgaricus*) and *Streptococcus salivarius* subsp. *thermophilus* (*Str. thermophilus*) which obtained from Chr. Hansen's Laboratories, Horsholm, Denmark. The strains of *Lactococcus lactis subsp. lactis* ATCC 11454 (*Lc. lactis* ATCC 11454) and *Lactobacillus acidophilus* JCM 1229 (*Lb. acidophilus* JCM 1229) used for manufacture of yoghurt to produce bacteriocin, also *Listeria monocytogenes* 1514 (*List. monocytogenes* 1514) used for determination of bacteriocin activity as indicator bacteria were obtained from Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

- Activation of cultures:

Lc. lactis ATCC 11454 was incubated at 30°C, *Lb. acidophilus* JCM 1229 was incubated at 37°C (two strains produced bacteriocins) and yoghurt starter culture was incubated at 40°C, during activation by three successive transfers in sterile 11% reconstituted skimmed milk powder (10^6-10^7cfu/ml). The active starter cultures were kept in refrigerator until use (through 24 hr, Badawi *et al.*, 2004). While, *List.*

monocytogenes 1514 were activated on tryptone soya broth (TSB) at 37°C/24 hr and reactivated twice (10^6 cfu ml^{-1}) and conserved in refrigerator (Abd El-Fattah, 1999).

- Yoghurt manufacture:

Some trials were conducted to know the ratio from *Lc. lactis* ATCC 11454 or *Lb. acidophilus* JCM 1229 which can be added with yoghurt starter to give ~100 AU/ml of bacteriocin activity in fresh yoghurt. The obtained results clear that the best ratio from every previous strains was 1%; when it is added alone with 1% yoghurt starter or 0.5% from every previous strains if it was added together with 1% yoghurt starter.

Yoghurt was manufactured according to Tamime (1978) from fresh mixed milk standardized to ~ 3% milk fat. It was heated to 85°C for 30 min, immediately cooled to 42°C and divided to eight portions (7 Kg each), and then inoculated with: 2% yoghurt starter cultures (control C1), 2 % yoghurt starter + 100 AU/ml of nisin, (control C2), 1% yoghurt starter + 1% *Lc. lactis* ATCC 11454 for producing ~100 AU/ml of bacteriocin (T1), 1% yoghurt starter + 1% *Lb. acidophilus* JCM 1229 for producing ~100 AU/ml of bacteriocin (T2), 1% yoghurt starter + 0.5% *Lc. lactis* ATCC 11454 + 0.5 % *Lb. acidophilus* JCM 1229 for producing ~100 AU/ml of bacteriocin (T3), 2% yoghurt starter + bacteriocin extract produced from *Lc. lactis* No.8, containing 100 AU/ml (T4), 2% yoghurt starter + bacteriocin extract produced from *Lb. acidophilus* No. 87, containing 100 AU/ml (T5) and 2% yoghurt starter + bacteriocin extract from *Lc. lactis* No. 8 (50 AU/ml) + bacteriocin extract from *Lb. acidophilus* No. 87 (50 AU/ml) (T6).

All treatments were put into yoghurt plastic cups (100 ml) and incubated at 42°C until the pH reached ~4.6 (coagulation time is recorded). Then, the treatments transferred to refrigerator and maintained at ~5°C. Yoghurts were analysed for the rheological, chemical, microbiological tests, bacteriocin activity and they were sensory evaluated when fresh and after 7, 14, 21 and 28 days.

- Methods of analysis:

- Chemical analysis:

Titratable acidity, total solids, fat, ash and protein contents of yoghurt treatments were determined according to the methodology mentioned by A.O.A.C, (1990). Lactose content was determined as suggested by the phenol-sulphuric method of Barnett and Abdel-Tawab (1957). Total volatile fatty acids (TVFA) contents were determined by the direct distillation method as described by Kosikowski (1984). Acetaldehyde content was determined according to the method described by Lees and Jago (1969). pH value of yoghurt samples was determined using a pH meter (JENCO Model 1671, USA)

- Bacteriocin activity:

Preparation of yoghurt samples for determination of bacteriocin activity was as follows. Samples were initially macerated in equal volumes of distilled water in a stomacher (Lab. blender 400) for 15 min. and heated to 80°C for 10 min. (Ryan *et al.*, 1996) and then, bacteriocin activity was assayed by the agar-well diffusion method of Tagg and McGiven (1971) using *List monocytogenes* 1514 as indicator bacteria, with some modifications (Tahara and Kanatani, 1996).

- Microbiological examinations:

Lactic acid bacteria (LAB); yeasts & moulds; coliforms were counted according to Elliker *et al.* (1956); IDF (1990) and A.P.H.A. (1992) respectively. While, *Lc. Lactis* ATCC 11454; *Lb. acidophilus* JCM 1229; *Lb. bulgaricus* and *Str. thermophilus* were counted as described by Ryan *et al.* (1996).

- Rheological analysis:

Curd firmness of yoghurt was measured using the Penetrometer Model Koehler Instruments Co., (USA) controller as described by Kammerlehner and Kessler (1980), the depth of penetration (0.1 mm = penetrometer unit) was measured after 5 sec at ~25°C (using cone weight 30 g and cone angle 45°C. The higher record by the penetrometer reading, the less firmness of yoghurt. Curd syneresis was determined according to the method of Dannenberg and Kessler (1988) with slight modification by Badawi *et al.* (2004).

- Sensory evaluation:

Yoghurt samples were evaluated organoleptically by 10 of the Staff Members of Food Science Department, Faculty of Agriculture, Moshtoher, Benha Univ., scoring was carried out as recommended by Mehanna *et al.* (2000).

- Statistical analysis:

Statistical analysis for the obtained data was carried out according to the method described by Clarke and Kempson (1997).

RESULTS AND DISCUSSION

Coagulation time:

The effect of bacteriocins produced or extracted by some LAB on the coagulation time of yoghurt (Table1) was significant ($P \leq 0.05$). It is obvious that the coagulation time of yoghurt showed highly significant increase (65.15%) by the addition of nisin in C2 ($P \leq 0.01$). Addition of bacteriocin extracts also significantly ($P \leq 0.05$) increased the coagulation time than that of the control and than that containing LAB which produce bacteriocins. The increase in coagulation time is due to the inibitory effect of nisin on the microorganisms and on the ability of producing acid which led to slow rate of acid development and long time of coagulation. (Olasupo *et al.*, 1996).

Rheological properties:

It could be observed (Fig 1) that there was no significant difference between treatments in curd firmness . This may be due to that the gross chemical composition in all treatments almost nearly the same in fresh yoghurt. This agree with Benech *et al.* (2003).

The curd firmness increased as the storage period extended for all treatments which may be attributed to the increase of total solids content during the storage (El-Nagar & Shenana 1998 and Ibrahim *et al.* 2004).

Table (1): Effect of bacteriocins on the coagulation time of yoghurt.

Treatments	Coagulation time	
	hr: min.	Increase %
C1	3:30	0.00
C2	5:45	65.15
T1	3.59	8.79
T2	3.40	3.03
T3	3.46	4.84
T4	4.27	29.39
T5	4.15	25.76
T6	4.20	27.27

C1 = Control (untreated)
C2 = Control with nisin
T1 = Yoghurt starter + *Lc. lactis* ATCC 11454
T2 = .. + *Lb. acidophilus* JCM 1229
T3 = .. + *Lc lactis* ATCC 11454 and *Lb. acidophilus* JCM 1229
T4 = .. + bacteriocin extract from *Lc. lactis* No. 8 (isolate)
T5 = .. + bacteriocin extract from *Lb. acidophilus* No. 87(isolate)
T6 = .. + bacteriocin extract from *Lc. lactis* No. 8and *Lb. acidophilus* No. 87 (isolates)

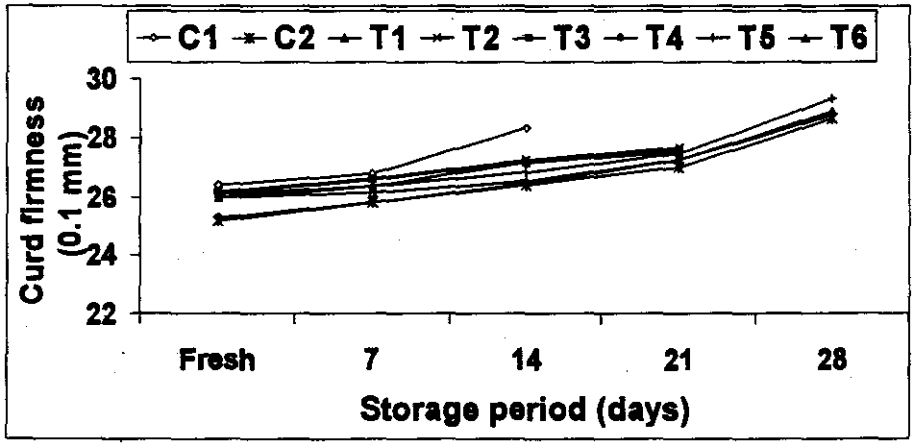

Fig. (1): Effect of bacteriocins on the curd firmness of yoghurt during storage.

Curd syneresis of the control (C1) was slightly higher than all treatments when fresh and throughout the storage period, while there was insignificant difference between treatments with bacteriocins (Table 2). These results may be due to the high acidity of control (El-Shibiny *et al.*, 1979). Meanwhile, curd syneresis of the all treatments slightly decreased during storage period.

Chemical composition of yoghurt:

Effect of bacteriocins produced or extracted from some LAB on the chemical composition of yoghurt during storage up to 28 days from different treatments are shown in Table (3).

Table (2): Effect of bacteriocins on the curd syneresis of yoghurt during storage period.

Treat-ments	Storage period (days)	Curd syneresis (g/100g)					
		15min	30min	45min	60min	90min	120min
C1	Fresh	17.71	24.29	28.83	31.81	35.89	39.96
C2		15.23	22.55	26.57	29.82	33.89	37.93
T1		16.53	23.98	27.66	31.12	34.57	38.36
T2		16.91	24.16	28.29	31.53	35.52	39.64
T3		16.89	24.00	27.90	31.28	34.77	38.54
T4		15.85	23.50	26.87	30.92	34.00	38.07
T5		16.32	23.97	27.07	30.99	34.45	38.27
T6		15.96	23.79	26.97	30.95	34.03	38.19
C1	7	17.51	23.92	28.44	31.61	35.65	39.51
C2		15.19	22.36	26.19	29.55	33.40	37.61
T1		16.29	23.62	27.45	30.41	34.40	38.09
T2		16.69	23.80	28.00	30.70	35.31	39.32
T3		16.44	23.65	27.56	30.69	34.56	38.13
T4		15.77	23.15	26.65	29.55	33.77	37.71
T5		16.10	23.59	26.99	29.91	34.11	37.96
T6		15.99	23.39	26.76	29.81	33.93	37.80
C1	14	16.97	23.64	28.29	31.24	35.48	39.19
C2		14.19	21.74	25.96	29.13	32.15	36.73
T1		15.55	22.44	27.32	30.15	33.00	37.66
T2		16.35	23.41	28.12	30.52	35.20	38.99
T3		15.78	22.71	27.49	30.37	33.24	37.95
T4		14.49	21.93	26.10	29.30	32.35	36.94
T5		15.46	22.25	26.99	29.97	32.94	37.43
T6		14.96	22.03	26.41	29.63	32.65	37.15
C2	21	13.94	21.00	25.34	29.08	31.85	36.05
T1		15.13	22.00	26.82	29.83	32.82	37.33
T2		16.13	23.17	28.06	30.23	33.90	37.82
T3		15.48	22.16	27.13	30.00	32.91	37.45
T4		14.27	21.26	25.86	29.10	32.04	36.25
T5		15.06	21.94	26.22	29.54	32.41	37.10
T6		14.63	21.51	26.03	29.40	32.23	37.07
C2	28	13.61	20.63	25.00	28.73	31.32	35.00
T4		14.26	21.00	25.84	29.00	31.81	35.23
T5		14.83	21.88	26.11	29.33	32.20	36.62
T6		14.62	21.35	26.04	29.16	32.07	35.88

C1 = Control (untreated) *C1 spoiled after 14 days - **T1, T2 and T3 spoiled after 21 days**
C2 = Control with nisin
T1 = Yoghurt starter + *Lc. lactis* subsp. *lactis* ATCC 11454
T2 = " + *Lb. acidophilus* JCM 1229
T3 = " + *Lc. lactis* subsp. *lactis* ATCC 11454 and *Lb. acidophilus* JCM 1229
T4 = " + bacteriocin extract from *Lc. lactis* subsp. *lactis* (isolate)
T5 = " + bacteriocin extract from *Lb. acidophilus* (isolate)
T6 = " + bacteriocin extract from *Lc. lactis* subsp. *lactis* and *Lb. acidophilus* (isolates).

Table (3): Effect of bacteriocins on the chemical composition of yoghurt during storage period.

Treatments	Storage period (days)	T.S. %	Fat %	Protein %	Ash %	Acidity %	Lactose %	TVFA (0.1NaOH/100 g)	Acetaldehyde (µg/100 g)
C1	Fresh	13.075	3.190	3.991	0.895	0.790	4.360	16.930	77.560
C2		13.134	3.180	4.033	0.909	0.627	4.652	15.400	50.000
T1		13.060	3.170	4.024	0.903	0.710	4.492	16.050	72.300
T2		13.098	3.180	4.010	0.908	0.761	4.442	16.430	75.460
T3		13.059	3.200	4.004	0.905	0.740	4.483	16.100	74.330
T4		13.105	3.180	4.019	0.897	0.642	4.589	15.766	55.666
T5		13.099	3.180	4.032	0.916	0.680	4.489	15.900	58.500
T6		13.112	3.180	4.028	0.899	0.671	4.551	15.800	56.550
C1	7	13.220	3.160	3.889	0.911	1.070	4.002	20.910	64.560
C2		13.252	3.160	4.023	0.918	0.730	4.301	17.600	45.866
T1		13.194	3.150	4.000	0.916	0.890	4.090	18.830	59.880
T2		13.188	3.160	4.001	0.919	0.957	4.103	19.060	62.530
T3		13.224	3.180	3.982	0.918	0.920	4.100	18.930	61.600
T4		13.240	3.170	4.010	0.913	0.772	4.280	17.733	49.766
T5		13.225	3.160	4.021	0.925	0.820	4.215	18.033	51.300
T6		13.235	3.160	4.016	0.906	0.800	4.241	17.966	50.500
C1	14	13.331	3.080	3.753	0.918	1.330	3.085	21.601	54.100
C2		13.391	3.130	4.005	0.931	0.820	3.924	19.266	42.800
T1		13.333	3.110	3.969	0.925	1.020	3.730	21.260	46.366
T2		13.345	3.110	3.969	0.932	1.160	3.693	21.530	53.100
T3		13.339	3.120	3.934	0.938	1.115	3.703	21.300	48.660
T4		13.374	3.130	3.989	0.925	0.892	3.899	19.600	42.950
T5		13.556	3.120	3.994	0.935	0.926	3.889	20.100	45.200
T6		13.374	3.130	4.000	0.926	0.910	3.890	19.933	44.533
C2	21	13.497	3.100	3.963	0.947	0.912	3.546	21.661	37.160
T1		13.477	3.070	3.926	0.933	1.176	3.400	23.250	42.466
T2		13.469	3.070	3.918	0.942	1.289	3.294	23.751	44.530
T3		13.483	3.070	3.910	0.942	1.238	3.307	23.370	42.833
T4		13.476	3.090	3.948	0.934	1.050	3.524	21.833	39.166
T5		13.482	3.080	3.954	0.947	1.099	3.500	21.933	42.260
T6		13.470	3.100	3.961	0.936	1.060	3.517	21.866	39.933
C2	28	13.617	3.050	3.941	0.963	1.070	3.273	23.500	26.133
T4		13.585	3.040	3.916	0.969	1.170	3.206	23.633	31.466
T5		13.581	3.050	3.922	0.964	1.263	3.192	23.900	39.833
T6		13.617	3.050	3.927	0.957	1.243	3.190	23.866	36.700

C1 = Control (untreated) *C1 spoiled after 14 days - T1, T2 and T3 spoiled after 21 days
C2 = Control with nisin
T1 = Yoghurt starter + *Lc. lactis* subsp. *lactis* ATCC 11454
T2 = " + *Lb. acidophilus* JCM 1229
T3 = " + *Lc. lactis* subsp. *lactis* ATCC 11454 and *Lb. acidophilus* JCM 1229
T4 = " + bacteriocin extract from *Lc. lactis* subsp. *lactis* (isolate)
T5 = " + bacteriocin extract from *Lb. acidophilus* (isolate)
T6 = " + bacteriocin extract from *Lc. lactis* subsp. *lactis* and *Lb. acidophilus* (isolates).

A slight or no effect could be observe on the total solids, fat, protein and ash contents among the different treatments of the same age. These results in agreement with (Akalin, (1996) and El-Nagar & Brennan, 2001).

Addition of nisin directly to yoghurt milk (C2) was significantly decreased ($P \leq 0.05$) the acidity development and the lactose fermentation rate compared with control yoghurt (C1). A narrow effect was observed in other bacteriocin treatments. Bayoumi (1991) stated that the addition of nisin or bacteriocins retarded growth of lactic acid bacteria, thereby delaying acid production of yoghurt. Titratable acidity increased and lactose content decreased during the storage period in all treatments which may be due to the ability of lactic acid bacteria to convert lactose to i.e. lactic acid, acetaldehyde and acetoin (El-Shibiny *et al.*, 1979).

T.V.F.A were slightly higher in C1 followed by T2, T3, T1, C2, T4, T6 and T5. This may attributed to the higher effect of nisin and bacteriocin extracts on the growth of yoghurt starter (Gupta & Prasad, 1989 and Bayoumi, 1991). During storage, T.V.F.A. gradually increased ($P \leq 0.05$) in all treatments until the end of shelf life. The increase in T.V.F.A may be due to several lipases and esterases (Gupta & Prasad 1989 and Benech *et al.*, 2003).

The analysis of variance for acetaldehyde between treatments during storage was significant ($P \leq 0.05$). The maximum content of acetaldehyde was belonged to the control (C1) which was free from bacteriocins, while the minimum amount was belonged to (C2) which contains nisin as its effect on the biochemical changes was more pronounced than other treatments. Addition of nisin retarded the growth of lactic acid bacteria, thereby delayed acid and acetaldehyde production (Bayoumi, 1991).

Acetaldehyde content decreased significantly ($P \leq 0.05$) during storage progress until the end of the shelf life in all treatments. This may be due to the demonstrated ability of numerous lactic acid bacteria to convert the acetaldehyde to ethanol (Gupta & Prasad 1989).

Microbiological analysis of yoghurt:

Effects of bacteriocins on the microbiological counts of fresh yoghurt and during storage of different treatments are shown in Fig. (2). The lactic acid bacterial counts of the control yoghurt (C1) were higher than all treatments in fresh or stored yoghurt. The maximum effect on the bacterial growth was detected for nisin-treated yoghurt (C2) followed by bacteriocin extracts-treated yoghurt (T4, T6 and T5) and lastly, the bacteriocins produced by cultures (T1, T3 and T2). This attributed to the bactericidal or bacteriostatic effect of nisin and bacteriocins on the microorganisms (Bayoumi, 1991and Kebary & Kamaly, 1991). It is obvious that the changes in the counts of lactic acid bacteria of yoghurt from different treatments increased up to 7 days of storage and then decreased.

Nisin and bacteriocin treated yoghurt retarded growth of *Str. thermophilus* and then the counts were declined until the end of storage period. The effect was at its maximum for treatments C2, followed by T4, T6, T5, T2, T3 and lastly T1. Also, *Str. thermophilus* counts took the same trend as it enumerated the lowest for control

yoghurt (C1) when fresh and during storage period. This may be due to sensitively of these strains to the produced acidity (Kebary & Kamaly, 1991 and El- Nagar & Shenana, 1998).

Nisin and bacteriocin treated yoghurts showed a reduction in counts of *Lb. bulgaricus* compared to that in the control yoghurt (C1) when fresh or throughout the storage period. However, there was an increase in counts of *Lb. bulgaricus* for all treatments after 7 days of storage, but the increase rate was higher in control yoghurt (C1) than all treatments and then declined until the end of the storage period.

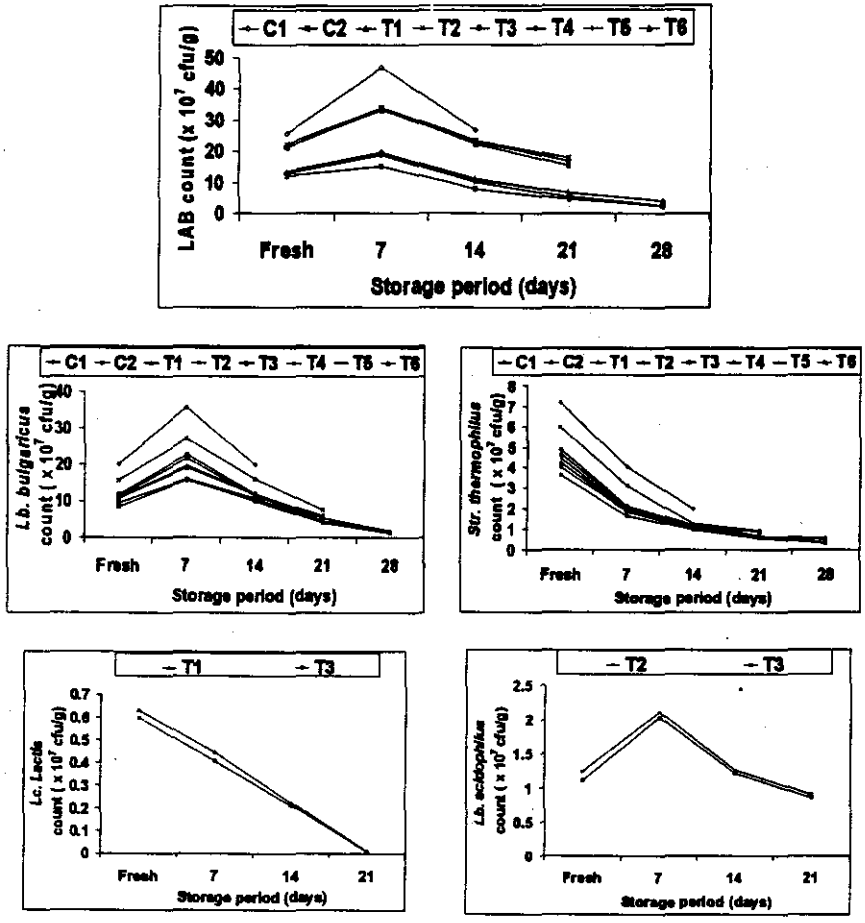

Fig. (2): Effect of bacteriocins on microbiological quality of yoghurt during storage period.

Kebary & Kamaly (1991) and El-Nagar & Shenana (1998) reported similar results. These differences in the inactivation rate of *Str. thermophilus* and *Lb. bulgaricus* in the presence of bacteriocins can be attributed to different sensitivities of the type of organisms to bacteriocins or due to nisin inactivation by nisinase

associated with nisin- resistant strains (Alifax and Chevalie, 1962). Also, This may be attributed to the increase of acidity which affects streptococci while, lactobacilli tolerate (El- Nagar and Bernnan, 2001). On the other hand, *Lb. bulgaricus* count was higher than *Str. thermophilus* when fresh and during storage.

The *Lc. lactis* ATCC 11454 counts (T1 and T3) were declined until the end of storage period in all treatments, while the *Lb. acidophilus* JCM 1229 counts (T2 and T3) increased during the first 7 days of storage and then declined till the end of storage period.

Coliform bacteria and yeasts & moulds were not detected in all yoghurt treatments either fresh or stored which is due to the high hygienic conditions during the preparation and storage of yoghurt. Also, it may be due to the role of lactic acid bacteria in preservation of the product which associated with their ability to produce some antimicrobial compounds (El- Nagar & Shenana, 1998 and Ibrahim *et al.*, 2004).

Nisin and bacteriocin activity:

Activity of nisin and bacteriocin produced or extracted by LAB in yoghurt treatments when fresh and during storage are shown in Table (4). The results reveal that yoghurt control (C1) was free from bacteriocins when fresh and during storage. The activity of nisin (C2), bacteriocin produced (T1) and bacteriocin extracts (T4, T5 and T6) were ~100 AU/ml in yoghurt when fresh and decreased ($P \leq 0.05$) during the storage in all treatments. This may be attributed to the action of protoelytic enzymes or nisinase from lactic acid bacteria, which may act on nisin peptides resulting in the degradation of the active peptide (Benech *et al.*, 2003). Concerning the activity of bacteriocin for treatments T2 and T3, there was a slight increase ($P \leq 0.05$) during the first 7days of storage. This may be due to that the count of *Lb. acidophilus* (bacteriocin-producer) in these treatments increased during the first 7 days of storage and then declined till the end of the storage period. (Benech *et al.*, 2003).

Organoleptic properties:

Organoleptic properties of yoghurt treatments including flavour, appearance and body & texture are presented in Fig (3). Results indicate variations in flavour and appearance between the control yoghurt (C1) and the yoghurt made with nisin (C2) or other bacteriocins ($P \leq 0.05$). These variations are due to the effect of the treatments on the activity of yoghurt starter and it was more pronounced in the nisin and bacteriocin extracts than the bacteriocin produced by LAB. After 14 days of storage, the control yoghurt (C1) gained minimum points for total scores. After 21 days of storage, yoghurt prepared with bacteriocin extracts (T4, T6 and T5 in sequence) got the highest scores, followed by yoghurt made with nisin (C2), then yoghurt made with bacteriocins produced by LAB (T1, T3 and T2 consecutively). This may be due to the increase of acidity which affects the rheological and sensory properties. After 28 days of storage, yoghurt made with bacteriocin extract (T4) recorded the maximum points of total scores followed closely by yoghurt made with nisin (C2). Wheares yoghurt prepared with bacteriocin extracts (T6 and T5) came after in total scores. The

differences between treatments were significant ($P \leq 0.05$). Nisin-treated yoghurts (~100 AU/ml) has good appearance and acceptable flavour, however nisin-treated yoghurts < 100 AU ml-1 gave a weak consistenency (Bayoumi, 1991). This result confirmed with Gupta & Prasad (1989); Olasupo *et al.* (1996) and Ibrahim *et al.* (2004).

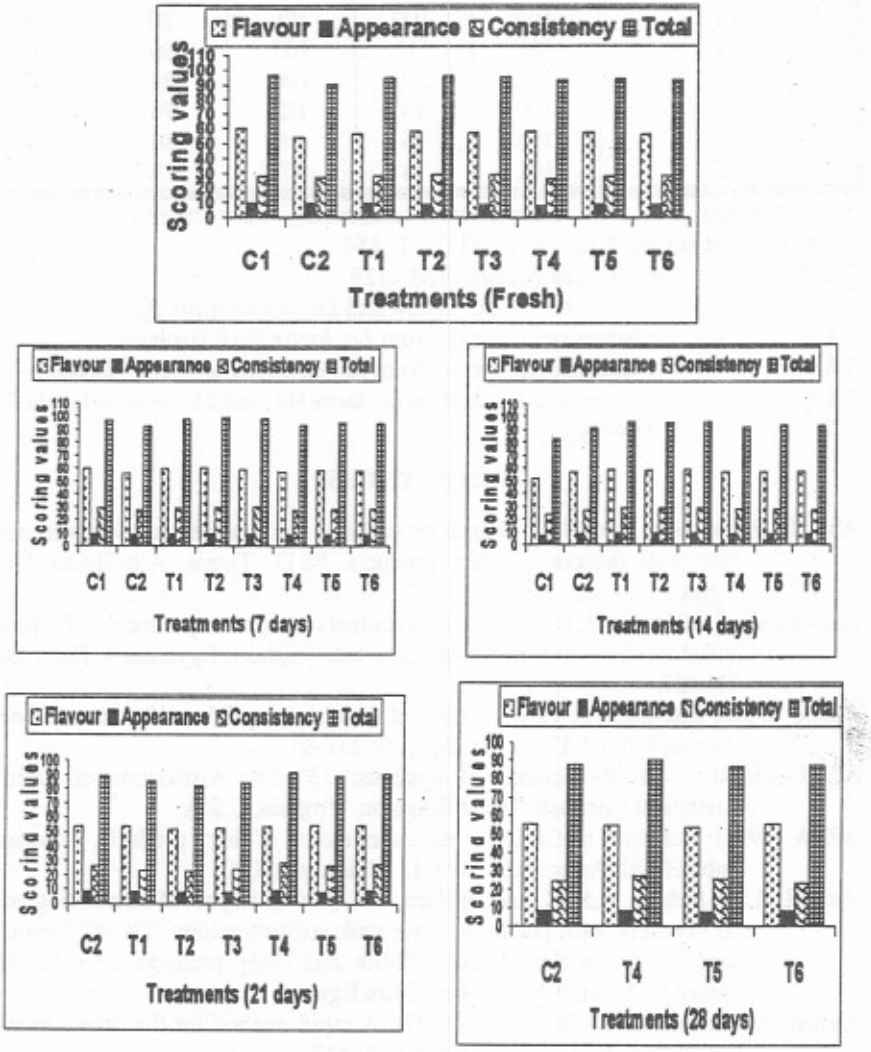

Fig. (3): Effect of bacteriocins on the sensory evaluation of yoghurt during storage period.

From the foregoing results, it can be recommend that addition of bacteriocins extracted or nisin (conc. ~100 AU/ml to milk could produce yoghurt with good organoleptic, rheological properties and with a prolonged shelf life.

Table (4): Bacteriocins activity in yoghurt during storage period.

Treatments	Bacteriocin activity (AU / ml)				
	Fresh	7 days	14 days	21 days	28 days
C1	0.0	0.0	0.0	S*	S
C2	98	92	85	80	73
T1	107	100	90	80	S
T2	103	115	105	90	S
T3	102	110	100	88	S
T4	105	95	82	76	70
T5	106	96	88	80	74
T6	101	94	87	79	70

S** = Spoiled C1 = Control (untreated) C2 = Control with nisin

T1 = Yoghurt starter + *Lc. lactis* ATCC 11454

T2 = " " + *Lb. acidophilus* JCM 1229

T3 = " " + *Lc lactis* ATCC 11454 and *Lb. acidophilus* JCM 1229

T4 = " " + bacteriocin extract from *Lc. lactis* No.8 (isolate)

T5 = " " + bacteriocin extract from *Lb. acidophilus* No.87(isolate)

T6 = " " + bacteriocin extract from *Lc. lactis* No.8and *Lb. acidophilus* No.87 (isolates)

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إستخدام البكتريوسينات المنتجة بواسطة بعض بكتريا حامض اللاكتيك كمادة حافظة طبيعية في الزبادي

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قيم تأثير البكتريوسينات المنتجة في الزبادي من السلالات

Lactococcus lactis subsp. *Lactis* ATCC 11454 (T1); *Lactobacillus acidophilus* JCM 1229 (T2); *Lactococcus lactis* subsp. *lactis* ATCC 11454 &*Lactobacillus acidophilus* JCM 1229 (T3)

ومستخلصات البكتريوسينات المضافة للزبادي للسلالات

Lactococcus lactis subsp. *lactis* isolate (T4); *Lactobacillus acidophilus* isolate (T5); *Lactococcus lactis* subsp. *lactis* & *Lactobacillus acidophilus* isolates (T6)

كمواد حافظة طبيعية في الزبادي مقارنة بزبادي الكنترول (C1) والزبادي المعامل بالنيسين (C2). واستخدم النيسين والبكتريوسينات المنتجة ومستخلصات البكتريوسين بتركيز ~ ١٠٠وحدة نشاط/ مل. فحصت عينات الزبادي لوقت التجبن والتحليلات الكيماوية والخصائص الريولوجية والجودة الميكروبيولوجية ونشاط البكتريوسين والتقييم الحسي ومدة الصلاحية خلال فترة التخزين حتي ٢٨ يوم / ~ ٥ م. أدت معاملة الزبادي بالنيسين أوالبكتريوسينات المنتجة أوالمستخلصة من بعض سلالات بكتريا حامض اللاكتيك إلي زيادة وقت التجبن مقارنة بزبادي الكنترول. كما أدي وجود البكتريوسينات إلي تأخير نموبكتريا حامض اللاكتيك وبالتالي انخفاض الحموضة، بينما كانت الحموضة مرتفعة في زبادي الكنترول وخلال التخزين. حدث تحسن في الخصائص الريولوجية والحسية ومدة الصلاحية للزبادي المعامل بالنيسين أوالبكتريوسينات المستخدمة. وظلت جودة ومدة صلاحية الزبادي المحتوي علي مستخلصات البكتريوسين والنيسين مقبولة حتي ٢٨ يوم تخزين (T4 ، C2 ، T6 و T5 علي التوالي) يليها بعد ٢١ يوم تخزين الزبادي المحتوي علي البكتريا المنتجة للبكتريوسين (T1، T3 وT2 علي التوالي) مقارنة بعد ١٤ يوم لزبادي الكنترول. ويستنتج من ذلك أنة بإضافة مستخلص البكتريوسينات أوالنيسين (بتركيز ~ ١٠٠وحدة نشاط/ مل) يمكن إنتاج زبادي له خصائص حسية جيدة مع إطالة مدة الصلاحية.