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Effect of Using Probiotic Bacteria as Adjunct Culture in Domiati Cheese Making

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Abstract

The composition and quality of probiotic Domiati cheese were studied during 90 days of ripening in brine. Six cheese treatments were made using different types of cultures, which are: C (Control): *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*, T1: as control + *Lactobacillus casei*, T2: as control +*Lactobacillus acidophilus*, T3: as control + *Lactococcus lactis* biovar *diacetylactis*, T4:*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and T5:*Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Streptococcus thermophilus*. A gradual decrease in moisture content was observed as pickling proceeded. Titratable acidity, salt and fat content continuously increased as storage proceeded in all treatments. The soluble nitrogen and amino nitrogen were higher in the probiotic cheese, compared with the control. Enumeration of lactic acid bacteria (LAB) increased up to 45 days of storage and started to decrease with prolonging the storage period. The highest numbers of total bacteria and LAB were detected in T1 and T4, respectively, at the end of the storage, while the lowest was found in T5and T3, respectively. All fresh cheese treatments were found completely free of yeasts and moulds, while it started to appear after 15 days of storage. Absence of coliform bacteria was detected in all treatments. Higher overall scores were achieved in all treatments with added probiotic bacteria compared with control at the end of the storage period. Using probiotic bacteria as adjuncts enhanced the quality and improved the organoleptic properties of the resultant cheese.

Keywords: *Domiati cheese; probiotic bacteria; chemical composition; organoleptic properties.*

Introduction

The trend of using probiotic bacteria in making dairy products was noticed during the last few years, because of their health benefits. These dairy products could be considered functional foods, which have positive effects on health. The recommended concentration of probiotic bacteria to provide its therapeutic health benefits is 10^6cfu/gm of the product when consumption (Gomes *et al.*, 2011).

Cheese has certain potential advantages that provide a good alternative to fermented milk as a carrier of probiotic bacteria. Cheese components create a buffer against the highly acidic environment in the gastrointestinal tract, which is a more favorable environment for the survival of probiotics throughout gastric transit. Moreover, the relatively high-fat content of cheese may offer extra protection to probiotic bacteria in the

gastrointestinal tract (Stanton *et al.*, 1998; Gomes da Cruz *et al.*, 2009).

The selection of strains should be carefully evaluated to increase probiotic cell viability during cheese manufacture, as well as to limit potential changes in the sensory properties of cheese, especially in pickled type cheeses like Domiati cheese in Egypt (Yerlikaya and Ozer, 2014).

Domiati cheese is one of the most famous types of soft white cheese in Egypt. It is taken fresh or 3-6 months after the ripening period in a pickling solution. The salt concentration used in the manufacture of Domiati cheese has a wide range, from 2 to 15%, which is influenced by some factors such as the type of milk, the ripening period and the season (Mehaia, 1993).

Using probiotic bacteria in the making of cheese, especially lactobacilli, can lead to many changes in the sensory properties, such as hydrolysis of peptides into oligo-peptides and amino acids that affect the flavor, texture and texture of the cheese (Shihata and Shah, 2000; Souza and Saad, 2009).

The aim of this study was to investigate the possibility of making good quality probiotic soft cheese (Domiati type) using different probiotic cultures and to observe the changes in chemical composition, microbiological quality and sensory properties during three months of storage.

Materials and Methods

Buffalo milk was obtained from the morning milking of the herd of the Faculty of Agriculture, Assiut University, Assiut, Egypt. Rennet powder was obtained from Chr. Hansen Laboratory, Denmark. A good grade of table cooking salt (sodium chloride) was purchased from the local markets in Assiut city.

Pure cultures of *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactobacillus casei, Lactobacillus acidophilus, Lactococcus lactis biovar diacetylactis, Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Bifidobacterium bifidum* were obtained from the Egyptian Microbial Culture Collection at Cairo Microbiological Resources Center, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The obtained cultures were individually activated by the technique of the three successive transfers in sterilized 10% reconstituted skim milk.

- **Cheese manufacture**

Domiati cheese was made from standardized buffalo's milk (2.5% fat) as the method described by Mohammed Mohammed *et al.* (2016), with some modifications. Milk was heated momentarily to 73°C and cooled to 40°C. Immediately after that, milk was salted with 5% sodium chloride. The resultant mixture was divided into six portions in which the starter culture was added according to the different treatments and left for 30 minutes, then rennet was added and mixed well. The milk was maintained at 40 °C until coagulation. Next, the curd was scooped into a cheesecloth, drained for two days at 5°C., the cheese was then removed from the cheesecloth for pickling. The resultant cheese was pickled in the salted whey that obtained from the manufacturing process for three months. Cheese treatments were

sampled and analyzed for the chemical, microbiological and organoleptic properties when fresh and after 15, 30, 45, 60 and 90 days of storage. Each treatment analysis was carried out in triplicates.

Cheese treatments and analysis

Control cheese treatment (C) was made by using 2% *Lactococcuslactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* as a starter (1:1).

T1 was prepared by using 2% *Lactococcuslactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus casei* (1:1:1).

T2 was made by using 2% *Lactococcuslactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris* and *Lactobacillus acidophilus*(1:1:1).

T3 was made by using 2% *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* biovar *diacetylactis* (1:1:1).

T4 was made by using 2% *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (1:1).

T5-was made by using 2% *Lactobacillus acidophilus, Bifidobacterium bifidum* and, *Streptococcus thermophilus* (1:1:1).

Cheese samples were analyzed for moisture, titratable acidity, salt, fat, soluble nitrogen and amino nitrogen according to AOAC (2012).

Microbiological analyses were examined for total colony forming units (CFU) as FIL/IDF Standard 153 (1991)**,** lactic acid bacteria count as FIL/IDF Standard 117B (1997) and yeasts and moulds on potato agar medium according to FIL/IDF Standard 94A (1985). Counting of coliform bacteria was detected according to FIL/IDF Standard 73A (1985).

Sensory evaluation of the examined cheese samples was evaluated according to *El-Shafei et al*. (2008) by the staff members of the Dairy Science Department, Faculty of Agriculture, Assiut University. The samples were presented to the panelists in random order and evaluated for the flavor, body and texture, appearance and overall acceptability with 45, 35, 20 and 100 points, respectively.

Statistical Analyses:

A 2×2 factorial design with the type of starter and storage period as fixed factors was used. The experimental data were analyzed using the SAS system (SAS, 1999). The means were reported and differences were analyzed using Duncan's multiple range test and considered significant when $P \leq 0.05$(Steel and Torrie, 1980).

Results and Discussion

The mean moisture content of all treatments is presented in Table (1). Using of adjunct in making Domiati cheese resulted in an effect on the moisture content of fresh cheese treatments and throughout the ripening period. Higher moisture content was observed in T2, T3 and T5, compared with that in the C, T1 and T4 ($P \leq 0.05$). On the other hand, a gradual decrease ($P \leq 0.05$) in moisture content was detected in all treatments as the pickling period proceeded, which might be attributed to the shrinkage of the curd as a result of the development of acidity during the pickling period, which helps to eject the whey from the curd. Similar re-

sults were reported by Kebary and Youssef (2015).

Results obtained in Table (1) illustrate the titratable acidity of probiotic cheese during storage. An increase in acidity could be observed in all treated samples during the storage period (P≤0.05). Comparing different treatments when fresh and during the storage periods, the lowest acidity was found in C., however, the highest acidity was observed inT2and T3. Similar results were mentioned by Mahmoudi *et al.* (2012), who found a significant decline in the values of pH during the ripening of Iranian white pickled cheese and also he found higher acidity in the probiotic cheese containing starter of *Lb. acidophilus* than the control cheese. In another study by Ong *et al.* (2007) the authors found that the pH of probiotic cheddar cheeses was in general lower than that of control cheese where the acetic acid concentration increased in cheese with *Lb. casei*, *Lb. paracasei* and *Bifidobacterium sp.* during ripening.

Table 1. Means values of the chemical composition of Domiati cheese and their statistical evaluations in terms of the type of starter and storage period.

Property / Treatment	Moisture	Acidity	Amino nitrogen	Soluble nitrogen	Salt	Fat
C	61.80^{ab}	0.977	0.47^{b}	0.72^{b}	5.03	16.60
T1	61.42^{b}	0.987	0.48^{ab}	0.73^{ab}	5.07	16.40
T2	62.48^{a}	0.993	0.49^{a}	0.74^{ab}	5.07	16.67
T3	61.92^{a}	0.997	0.48^{ab}	0.75^{ab}	5.06	16.94
T4	60.95^{b}	0.978	0.48^{ab}	0.76^{a}	5.02	17.11
T5	62.58^{a}	0.983	0.48^{ab}	0.74^{ab}	5.05	17.00
Storage period (days)						
Fresh	67.37^{a}	0.82^{f}	0.19^{f}	0.43^{f}	4.38^{f}	12.83^{f}
15	63.83^{b}	0.90^{e}	0.20^{e}	0.50^{e}	4.63^{e}	14.22^{e}
30	61.86^{c}	0.95^{d}	0.53^{d}	0.69^{d}	5.11^{d}	16.00^{d}
45	60.58^{d}	0.99^{c}	0.55^{c}	0.78^{c}	5.22^{c}	17.33^{c}
60	59.67^{d}	1.10^{b}	0.70^{b}	0.87^{b}	5.33^{b}	19.06^{b}
90	57.97^{e}	1.15^{a}	0.71^{a}	1.18^{a}	5.62^{a}	21.33^{a}

Means within the same columns with different subscriptions are significantly different (P≤0.05). Means within the same columns without subscriptions are not significantly different (P>0.05). C (control): The type of starter is *Lactococcus lactis sub sp. lactis* and *Lactococcus lactis sub sp. cremoris,* T1: as control + *Lactobacillus casei*, T2: as control + *Lactobacillus acidophilus*, T3: as control + *Lactococcus lactis* biovar *diacetylactis*, T4: *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* and T5: *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Streptococcus thermophilus*

The effect of the type of starter on the amino nitrogen content of fresh cheese and throughout the storage period are presented in Table (1). The concentration of amino nitrogen in fresh cheese was almost similar, however during the storage period, there was a gradual increase in amino nitrogen content in all treatments, approximately by the same rate. Soluble nitrogen increased gradually ($P \leq 0.05$) in all treatments by the progress of the storage period (Table 2). Nitrogen fractions generally increase in cheese with the prolongation of the storage period, consistent with the continuous breakdown of casein and large peptides into amino acids and small peptides through the activity of starter culture enzymes and effects of the residual rennet (Lau *et al.*, 1991). The soluble nitrogen content was higher in the probiotic cheese treatments than the control cheese at the end of storage (Table 1). These results are in agreement with those obtained by (Kumar *et al.*, 2015), who detected a general increase of insoluble proteins in treated samples of Feta cheese made by using adjunct culture, compared with the control (in the absence of adjunct culture) during storage.

Salt concentration increased in all samples by prolonging the storage period ($P \leq 0.05$) as observed in Table (1). However, there were insignificant differences ($P \geq 0.05$) in the salt content between treatments. The increase in salt concentrations as the storage period is prolonged might be due to the decrease in moisture content, and the increase in the acidity of the cheese, which could be due to the convert of residual lactose in cheese to lactic acid, and the formation of varying concentrations of short-chain fatty acids as a metabolic end-product of the probiotic bacteria. Similar results were obtained by Bakirci *et al.* (2011).

Concerning the fat content in all treatments (Table 1**)**, it could be observed that the use of adjunct culture in making the probiotic cheese did not affect the fat content of the cheese. However, a gradual increase in fat content was observed up to the end of the storage of cheese. This can be referred to the decrease in moisture content of cheese. Similar results were found by Mahmoud *et al.* (2013).

Microbiological analyses of cheese samples during storage are presented in Fig. (1). The total bacterial count (CFU) in cheese samples was different between treatments and increased during the storage period up to 45 days of storage. It started to decrease up to 90 days of storage. At the end of the storage period, the highest CFU was found in T1, while the lowest CFU was found in T5. This result can be explained by the decrease in the viability of the species which are sensitive to the increase of the acidity. Similar results were observed by Mahmoud *et al.* (2013), who observed an increase of total bacterial counts in probiotic Karish cheese at the beginning of refrigerated storage, then decreased gradually up to the end of the storage period. Kasimo□lu *et al.* (2004) also found that the counts of aerobic mesophilic bacteria correlate with the count of the other Lactobacilli and Lactococci groups in Turkish white cheese made using a probiotic culture.

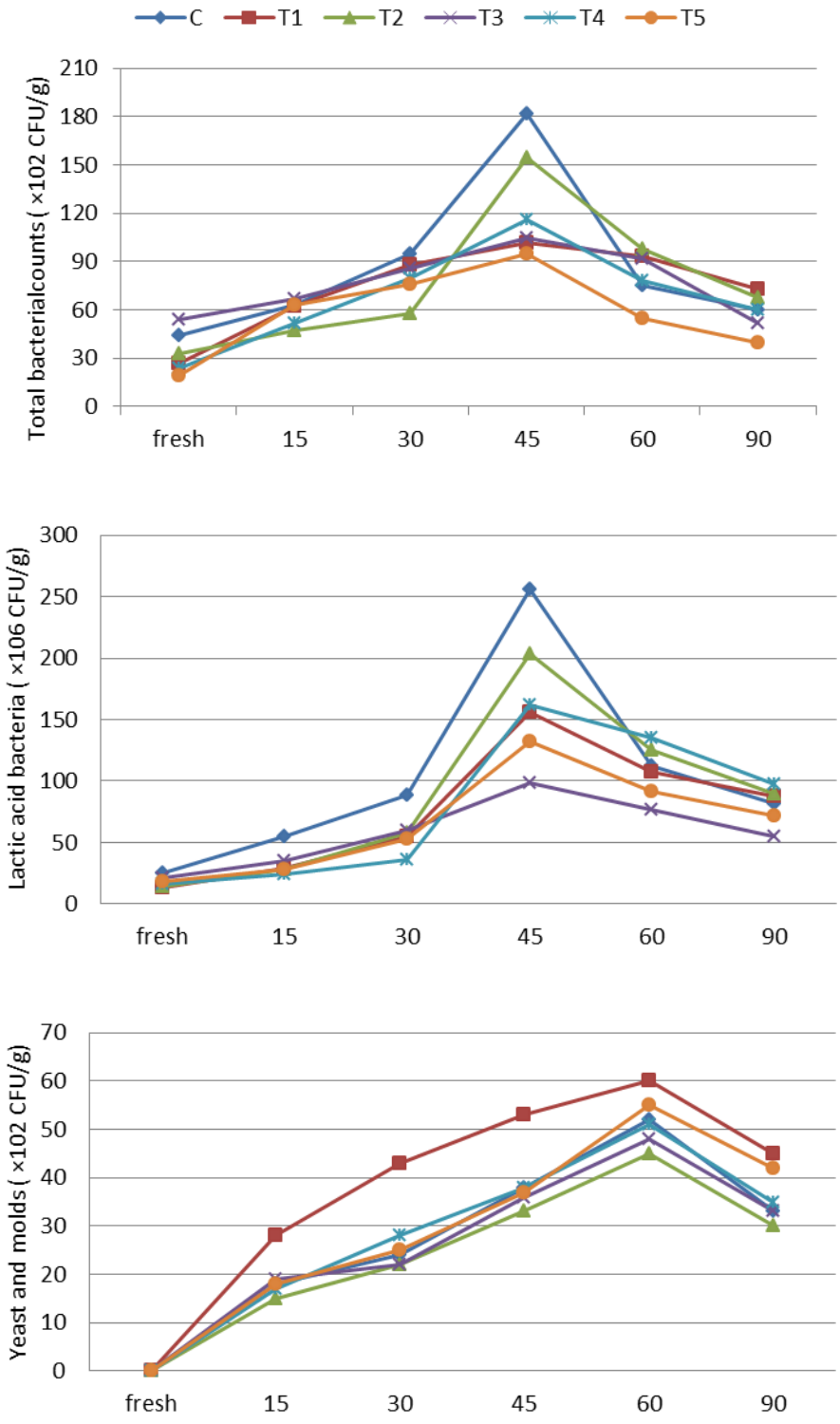

Fig. 1. Microbiological analysis of Domiati cheese made using different mixed cultures during 90 days of ripening. For used cultures in the different treatments see legend Table 1

Counts of lactic acid bacteria in cheese samples during storage are presented in Fig. (2). Results indicated that there was a gradual increase in the number of lactic acid bacteria, approximately by the same

rates, in all treatments up to 45 days of storage, followed by a gradual decrease in the counts of lactic acid bacteria up to 90 days of storage. The lowest numbers of lactic acid bacteria in fresh cheese was in T1 (13 x 10^6 CFU/g), while the highest was in C (25x10^6 CFU/g). T2, T4, T5 and T3 came in the middle, respectively. By the end of storage, the minimum count of the lactic acid bacteria was 55 x 10^6 CFU/g in T3 and the maximum was 98 x 10^6 CFU/g in T4, between these counts, T5, C, T1 and T2, respectively. These results could be explained by the increase in cheese acidity (Table 1). As the acidity can affect the viability of lactic acid bacteria. Kasimo□lu *et al.* (2004) explained the rapid growth of *L. acidophilus* in the first days of the ripening of Turkish white cheese made using a probiotic culture to the fermentation of lactose by starter Lactococci. Similar results were reported by Yerlikaya and Ozer (2014), who found a gradual increase in probiotic bacteria (above 10^6 CFU/g) throughout the storage of probiotic fresh white cheese. In addition, Stanton *et al.* (1998) claimed that *Lb. paracasei* was also satisfactorily viable during the long ripening period of Cheddar cheese.

Regarding the presence of yeasts and moulds in the different examined treatments during the storage period are presented in Fig. (1). The obtained results indicated that yeasts and moulds could not be detected in all fresh cheeses, and started to appear after 15 days of storage with a minimum count of 15x10^2 in T2 and a maximum count of 28x10^2 in T1, followed by a gradual decrease after 60 days of storage reaching the minimum of 30x10^2 in T2, and maximum of 45x10^2 in T1 at the end of storage period. These results came in harmony with those detected by Mahmoud *et al.*, (2013) in Probiotic Karish cheese, where the yeasts and moulds increased gradually during the storage period and reached their maximum by the end of the storage period for all cheese treatments. Concerning the coliform bacterial, it could be observed that these bacteria could not be detected in all treatments throughout the storage period, which is in agreement with the results of Kasimo□lu *et al.* (2004).

Table 2. Organoleptic properties of probiotic Domiati cheese during storage.

Sensory attributes	Treatments	Storage period (days)					
		Fresh	15	30	45	60	90
Flavor (45)	C	37.22	40.11	42.33	42.78	41.27	43.12
	T1	39.22	39.67	40.78	41.78	41.64	44.44
	T2	38.11	39.56	41.00	42.56	43.18	44.27
	T3	38.67	39.78	41.56	42.56	44.18	44.48
	T4	37.11	41.11	43.33	44.89	42.45	44.55
	T5	38.22	40.56	42.00	43.56	41.73	44.83
Body and Texture (40)	C	32.00	33.00	34.78	35.67	34.00	33.44
	T1	33.33	33.11	34.00	34.89	35.36	37.47
	T2	33.89	33.56	34.44	35.44	36.00	37.22
	T3	33.67	34.67	35.78	36.22	33.73	34.93
	T4	31.78	34.67	35.56	36.56	35.64	36.74
	T5	32.44	34.56	35.22	36.00	33.18	35.38
Appearance (15)	C	8.11	8.44	8.67	8.89	7.23	9.44
	T1	7.89	8.78	8.78	9.00	7.37	8.55
	T2	8.33	8.44	8.78	9.00	8.27	9.39
	T3	7.78	8.00	8.33	8.67	7.64	8.74
	T4	7.78	8.11	8.44	8.78	8.45	9.60
	T5	7.89	8.78	8.89	9.11	7.09	8.24
Overall Scores (100)	C	77.33	81.56	85.78	87.33	82.59	86.00
	T1	80.44	81.56	83.56	85.67	84.73	90.46
	T2	80.33	81.56	84.22	87.00	87.45	90.88
	T3	80.11	82.44	85.67	87.44	85.55	88.15
	T4	76.67	83.89	87.33	90.22	86.55	90.89
	T5	78.56	83.89	86.11	88.67	82.00	88.45

For used cultures in the different treatments see legend Table 1

Using probiotic bacteria in cheese making may affect the flavor and texture characteristics, and thus change the perceived acceptance of cheese by consumers. If organoleptic properties do not match consumer acceptance, the cheese will fail in the markets. In consideration of flavor, little difference in scoring was found in fresh Domiati cheese (Table 2), being a minimum of 37.11 in T4 and a maximum of 39.22 in T1. However, after 90 days of storage, the score of all the probiotic cheese was higher than the control. During cheese ripening, proteolysis is the most important flavour development pathway. Free amino acids and short peptides are essential for flavour development and depend on the extent of proteolysis (Kasimo□lu *et al.*, 2004). The body and texture, and appearance present the same trend as the flavor (Table 2). It is obvious that all samples were taken too close overall score on the first day of storage and T1, T2 and T3 gained an approximately close overall score. However, at the end of storage, the probiotic cheese gained a higher overall score than the control cheese. Between fresh and 90-day-old cheese, large differences in rating scores were observed among cheeses in all the sensory attributes (Table 2). These differences might be attributed to differences in protein fractions, whose levels showed the same trend of rat-

ing scores (see amino and soluble nitrogen content in Table 1).

Conclusion

The addition of probiotic bacteria as adjunct culture in Domiati cheese making leads to an increase in the amino nitrogen and soluble nitrogen contents, compared to the control. A gradual decrease in moisture as pickling proceeded was detected. However, titratable acidity, salt and fat content gradually increased as storage proceeded in all treatments. The total bacterial count and lactic acid bacteria increased at the beginning of ripening and started to decrease as the storage period was prolonged. Sensory analysis of the probiotic cheeses showed higher acceptability for experimental cheeses than for the control. Using probiotic bacteria as an adjunct culture could improve the quality and organoleptic properties of Domiati cheese.

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تأثير استخدام البكتيريا الداعمة للحيوية كبادئ مساعد في إنتاج الجبن الدمياطى

أسماء حسنى محمد منيب ، ياسر محمد عبد العزيز الدروى

قسم علوم وتكنولوجيا الالبان – كلية الزراعة – جامعة اسيوط

الملخص

تمت دراسة تركيب وجودة الجبن الدمياطى الحيوية خلال 90 يومًا من التخزين في محلول ملحي. تم إجراء ستة معاملات للجبن باستخدام أنواع مختلفة من البادئات وهى كالاتى C(الكنترول): *Lactococcus lactis* subsp. *lactis* و *Lactococcus lactis* subsp. *cremoris* و T1 مثل الكنترول + *Lactobacillus casei* و T2 مثل الكنترول + *Lactobacillus acidophilus* و T3 مثل الكنترول + *Lactococcus lactis* biovar *diacetylactis* و T4 : *Streptococcus thermophilus* و *Lactobacillus delbrueckii* subsp. *bulgaricus* و T5: *Lactobacillus acidophilus* و *Bifidobacterium bifidum* و *Streptococcus thermophilus* . كان هناك انخفاض تدريجي في الرطوبة مع تقدم فترة التخزين. تمت زيادة نسبة الحموضة والملح والدهن بشكل مستمر مع استمرار التخزين في جميع المعاملات. كان النيتروجين الذائب والنيتروجين الأميني أعلى في الجبن الحيوى مقارنة بالكنترول. زاد العدد الكلى للبكتريا (CFU) وبكتيريا حمض اللاكتيك (LAB) حتى 45 يومًا من التخزين ثم بدأت في الانخفاض مع تقدم فترة التخزين. في نهاية فترة التخزين كان أعلى قيمة للعدد الكلى للبكتريا وبكتريا حمض اللاكتيك في T1 وT4 ، على التوالي، والأدنى كان في T5 وT3 ، على التوالي. لم يتم العثور على الخمائر والفطريات في الجبن الطازج لجميع المعاملات بينما بدأت في الظهور بعد 15 يومًا من التخزين. لم يتم اكتشاف بكتيريا القولون في جميع المعاملات. اكتسبت جميع معاملات الجبن الحيوى درجة تقييم إجمالية أعلى من جبن الكنترول في نهاية فترة التخزين. استخدام البكتريا الداعمة للحيوية كبادئ مساعد يمكن أن يؤدي إلى تحسين الجودة والخصائص الحسية للجبن الدمياطى.