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ORIGINAL ARTICLES

Viability of *Lactobacillus paracasei* 441 produced by batch and continous biofilm reactor in some dairy products.

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ABSTRACT

Addition of *Lactobacillus paracasei* 441 improved the viability and keeping quality of cheese and gave pleasant taste, favorable acidity and increasing the nutritive value of resultant products. At that time the numbers of cells were still above the recommended threshold for probiotic effect. The survival of *L. paracasei* 441 produced from continous biofilm cultures till the end of storage could be attributed to that this organism was able to grow at high salt concentration and may this regarded as affects of biofilm tolerant effects. The set fermented milk manufactured with *L. paracasei* 441 had gained the highest total organoleptic scores compared to the control, this may be due to the effect of production of polysaccharides and improving the rheological properties of set fermented milk.

Key words: *Lactobacillus paracasei, viability, quality, cheese*

Introduction

Lactic acid bacteria are widely distributed in the nature. It plays as essential role in the production of all dairy products and is involved in the production of many other foods and drinks.

The first use of probiotic cultures to produce dairy products that claim health benefits as a part of the so called functional foods (Lankaputhra and Shah, 1995; Shah *et al*., 1995; El-Shibiny *et al*., 2005 and El-Baradei *et al*., 2006). Certain lactic acid bacteria , primary species of Lactobacilli and or bifidobacteria are included in a group referred to as probiotic bacteria.

Biofilm are defined as microbial cell layers, which are irreversibly or reversibly attached on solid surfaces. These attached cells are embedded in a self-produced exopolysaccharide matrix, and exhibited different growth and bioactivity compared with suspended cells with their high biomass density, stability, and potential for long-term fermentation (Demirci *et al*., 1993 and Demirci *et al*., 2007). Biofilm reactors are employed for the fermentation and bioconversion, which need large amount of biomass. The structure of the biofilm matrix contributes to the high resistance of microorganisms to the extreme conditions of pH temperature, and toxic substances (Norwood and Gilmour, 2000).

The aim of the present investigation is to study the viability of strain produced by batch and continous biofilm reactor in dairy products (white cheese and fermented milk).

Materials and Methods

1. Production of Lactobacillus paracasei 441 biomass starter:

a. Production of Lactobacillus paracasei 441 in batch culture:

The strain Lactobacillus paracasei 441 was produced by batch using basal medium in a close system without pH control at 37oC for 24hrs incubation.

b. Production of Lactobacillus paracasei 441 in continuous biofilm culture:

2. Production of white cheese using L. paracasei 441:

White cheese is produced according to Fahmi and Sharara (1950) from a heated of cow's milk (72°C/15 sec). Cheese milk was immediately cooled to 37°C and divided into 4 equal portions. The first portion (T1) was treated as a control. Then 1% starter (*lactobacillus paracasei* 441) was added and followed by rennet. Salt was

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added at a level of 0, 3, 6 and 10 % of milk in portion T1, portion T2, portion T3 and portion T4, respectively. The resultant cheeses were distributed into sterile plastic screw-cap containers and pickled in their own whey. Then, the plastic containers were tightly capped and stored at 4± 1°C and 20±1°C for two months. Cheese was evaluated bacteriology and organoleptically at intervals time of 0, 15, 30, 60 days of storage. Trials were carried out in triplicate.

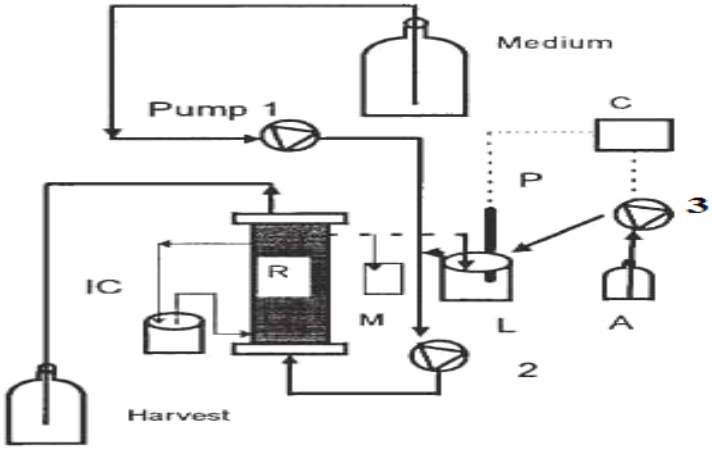

Fig. 1: Schematic diagram of the upflow packed-bed reactor. A, alkali; 1, feed pump for complete medium; 2, recycling pump; 3, alkali pump; C, pH controller; F, air filter; IC, heat exchanger; L, external pH controller device; M, sampling port; R, reactor; P, pH electrode.

3. Production of set fermented milk:

Fermented milk was manufactured according to the method of Tamime *et al.* (1995). Three batches of yoghurt were made from fresh cow's milk (3%) fat as follow:
T1: Cow milk + yoghurt starters "as control"
T2: Cow milk + yoghurt starters +*L. paracasei* 441 (produced by batch method)
T3: Cow milk + yoghurt starters +*L. paracasei* 441 (produced by continuous biofilm continuous culture)

Microbiological and chemical examination:

1. Titratable acidity and pH:

The acidity of cheese was determined using the method described by Ling (1963). The results were expressed as lactic acid percentage. Also, the pH of cheese was measured according to Ling (1963) using pH. Meter model Model 825 MP; Fisher Scientific Co., Pittsburgh, Pa., U.S.A.).

2. Lactobacilli count:

Lactobacilli count was determined using MRS agar according to (De Man *et al.*, 1960).The plates were incubated at 37º C for 48h under anaerobic condition.

Rheological Properties:

1. Syneresis:

Syneresis was determined by measuring the volume of separated whey (ml whey /100 ml yoghurt) collected after 30 min at room temperature 20^{o}C± 1 (Abd-El salam *et al.*, 1991)

2. Viscosity:

The viscosity of set yoghurt was measured using cylinder viscometer (Brookfield LVDV-II+PRO, with Rheocalc™ v3.0 software for automated instrument control and data acquisition.)

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3. Organoleptic assessment:

The Organoleptic properties of white cheese were assessed by a regular taste panel of the staff- members of the Dairy Science Department, National Research Center. White cheese samples were evaluated for flavor (50 points), body and texture (40 points) and appearance (10 points) according to Bodyfelt *et al.* (1988). On other hand the fermented milk organoleptic properties assessed according to Nelson and Trout, (1964).

Statistical analysis:

Statistical analysis and graphical representations were performed using Origin Lab Corporation software (Northampton, MA), ht

Results and Discussion

Changes in the viable counts of cheese starter Lactobacillus paracasei 441 during storage period:

Changes in the viable counts of all white cheese treatments with different salt concentrations (0, 3, 6 and 10%) respectively presented in Figs. (2, 3, 4 and 5). Data indicate the counts of these treatments gradually decreased along the storage period either at room or refrigerator temperatures. These results coincide with those stated by Mehanna *et al.(* 2002); Kebary *et al.* (2004) and Kebary *et al.* (2009). During storage, a slight decline in *L.paracasei* 441 numbers occurred in all treatments, during 60 days of storage, the cells decreased by an average of one logarithmic cycle (at 20^{o}C and at 4^{o}C). Depending on the source of cell production; batch or continuous biofilm cultures, we could notice that the viability and stability are higher in treatments with cell came from biofilm more than cells that came from batch. The introducing of starter produced by continuous biofilm increased the viable count by at least one log Figs. (3 and 5).

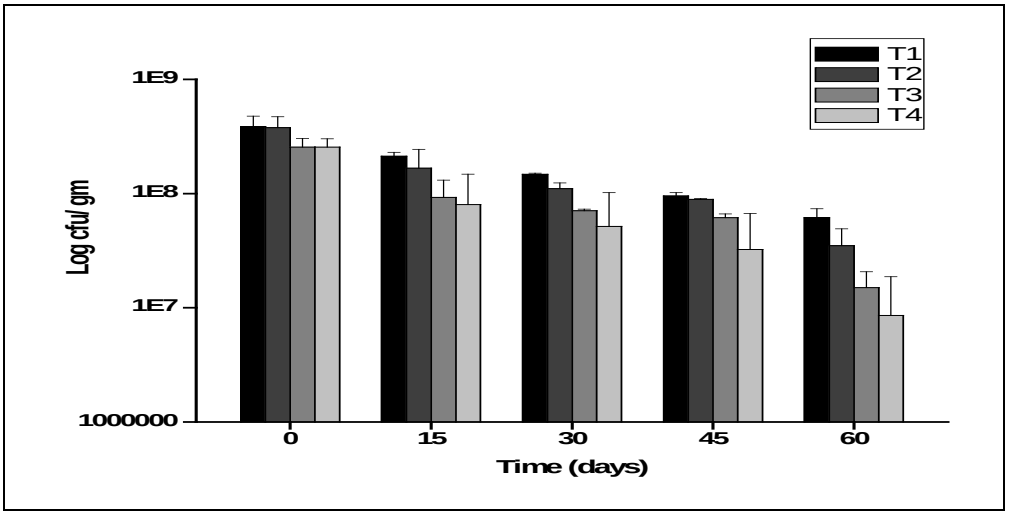

Fig. 2: Changes in the numbers of viable *Lactobacillus paracasei* 441 produced from batch culture in white cheese with different salt concentration during storage at refrigerator temperature (4^{o} C±1), Error bars indicate the Standard error (SE) of the mean of triplicates.

Generally, the survival of *L. paracasei* 441 produced from biofilm continuous cultures till the end of storage could be attributed to the fact that this organism was able to grow at high salt concentration (10%) and may thus be related to the effects of biofilm tolerant cells. In connection of these results similar pattern of tolerant effects of cells produced by biofilm was reported by Bruno Barcena *et al*. (1998); Bruno Barcena *et al.* (1999) and Bruno Barcena *et al.* (2001), in this respect, Dagher *et al.* (2010) reported in there review that immobilized cells have advantages over free cells, such as protection from toxic substances, increased plasmid stability and increased catalytic activity.

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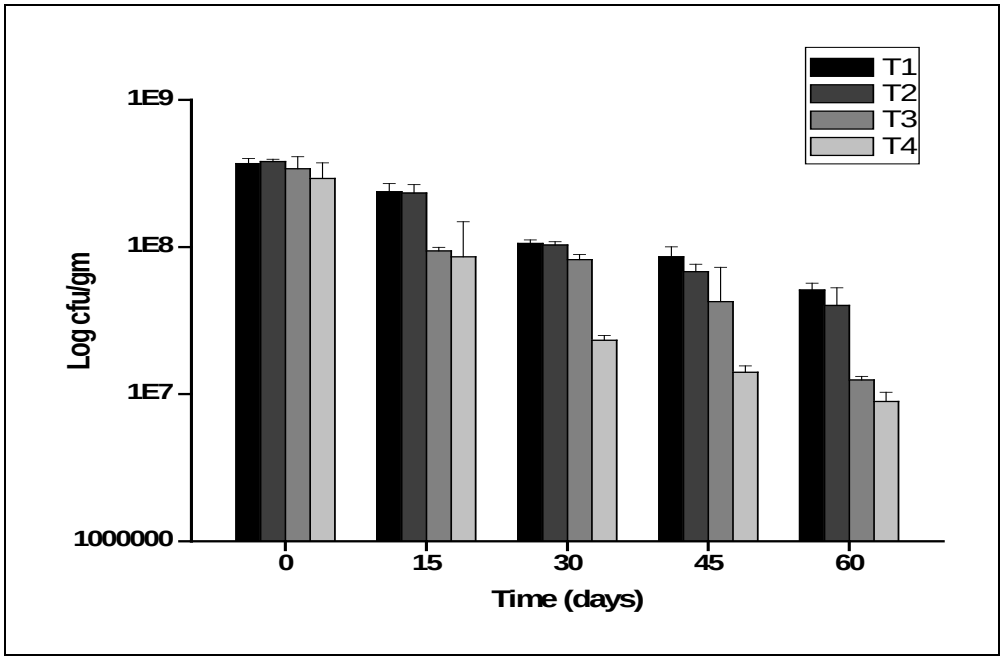

Fig. 3: Changes in the numbers of viable *Lactobacillus paracasei* 441 produced from biofilm continuous culture in white cheese with different salt concentration during storage at refrigerator temperature (4°C±1). Error bars indicate the Standard error (SE) of the mean of triplicates.

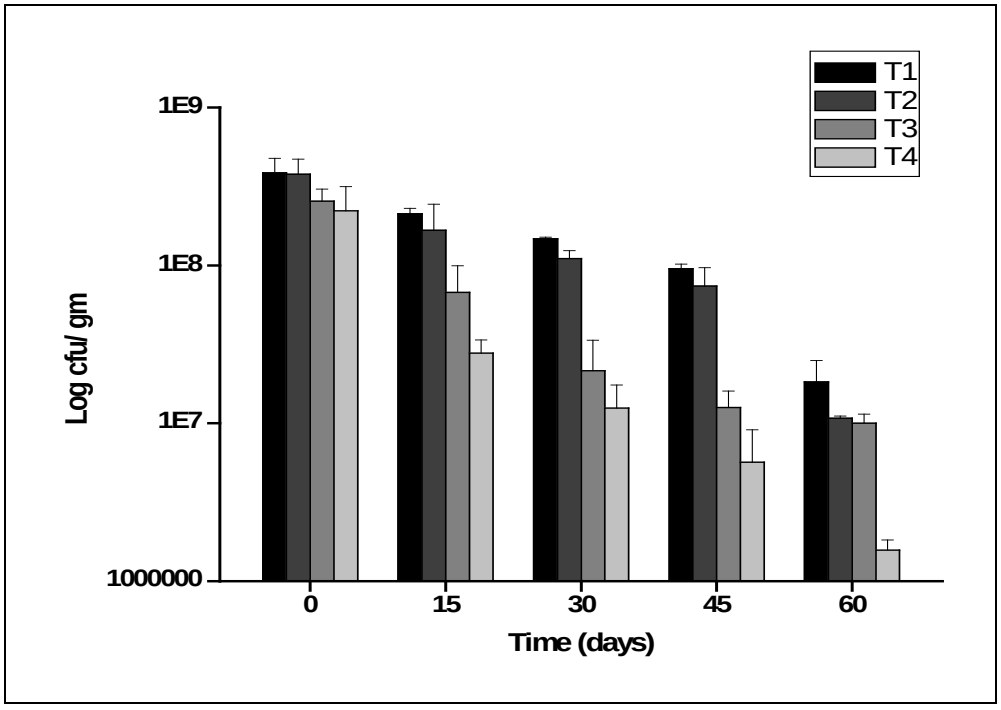

Fig. 4: Changes in the numbers of viable *Lactobacillus paracasei* 441 produced from batch culture in white cheese with different salt concentration during storage at room temperature (20°C±1). Error bars indicate the Standard error (SE) of the mean of triplicates.

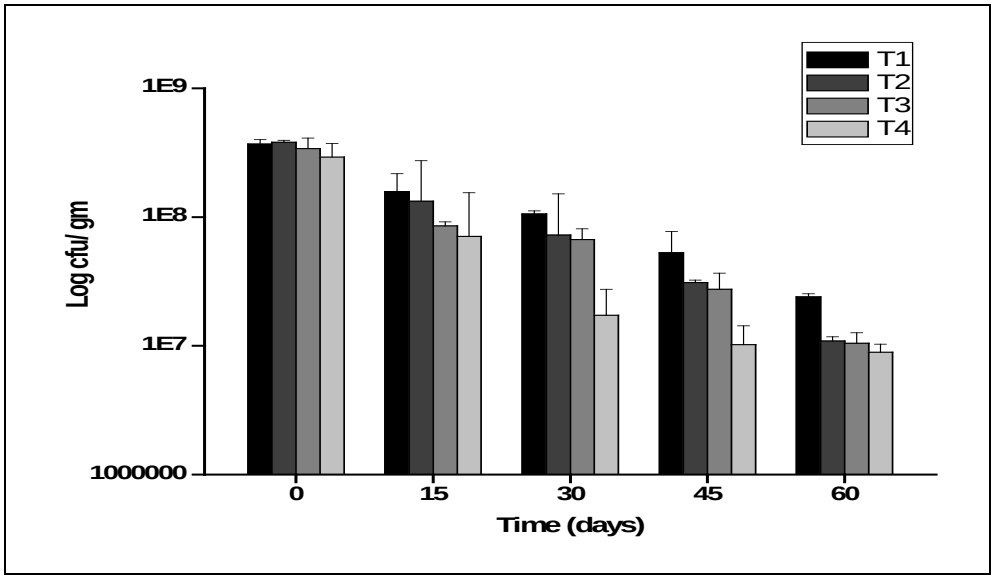

Fig. 5: Changes in the numbers of viable *Lactobacillus paracasei* 441 produced from biofilm continuous culture in white cheese with different salt concentration during storage at room temperature (20^{o}C±1). Error bars indicate the Standard error (SE) of the mean of triplicates.

Change in pH:

As shown in Figs. (6 and 7) pH values of cheese of different treatments gradually decreased throughout storage either at room or refrigerator temperature. This could be due to the formation of more acids during storage especially lactic acid as results of microorganisms' metabolism. These results are in agreement with Murad *et al.* (1998) and Effat (2000). Storage at room temperature (20^{o}C) increased lactose fermentation, which could explain the high decrease in the pH value of cheeses over those stored at low temperature. A similar trend was observed by Ibrahim and Deghedi, (1995); Effat (2000) and Ibrahim *et al*. (2003). There were no differences of pH between cheeses produced from cells came from batch or came from continuous biofilm system and there was no effect of salt concentration on pH values during storage period.

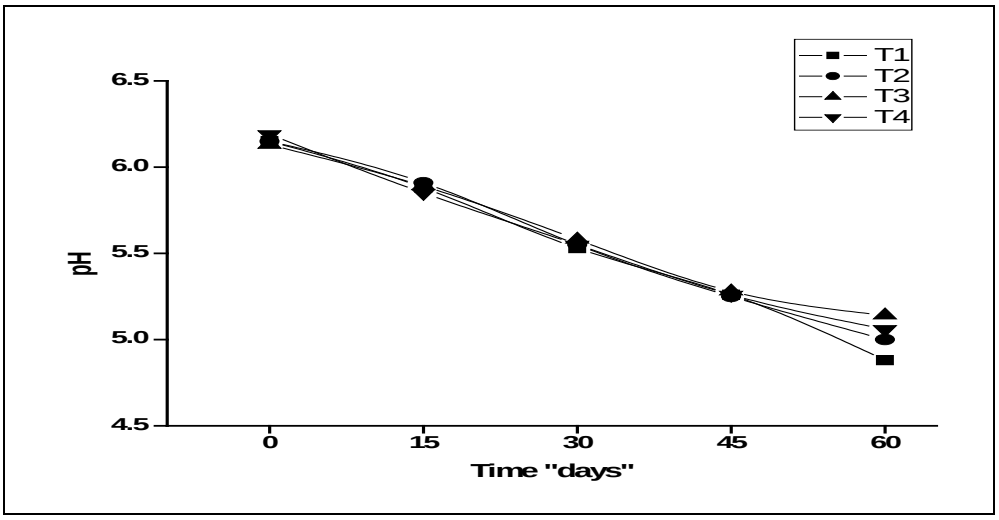

Fig. 6: Changes in the pH values of white cheese manufactured with different salt concentration during storage at room temperature 20^{o}C ±1.

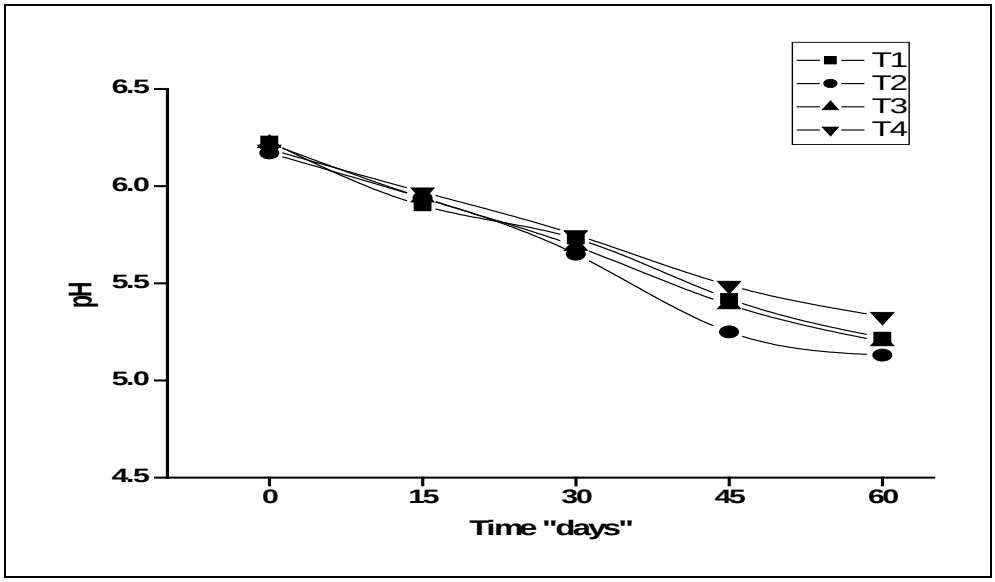

Fig. 7: Changes in the pH values of white cheese manufactured with different salt concentration during storage at refrigerator temperature 4°C±1.

Sensory evaluation of the cheese treatments:

The data presented in Table (1) show the average of sensory evaluation donated by the panelists.

It is evident from these results that the scores given to cheese made with different salt concentrations were generally higher than scores for control cheese. There was no evidence of effects on cells that came from batch or continuous culture in sensory evaluation. In conclusion the acceptability of previous cheese treatments could be ranked as follows treatments T3>T2>T4>T1 at room temperature and ranked as follows T4>T3>T2>T1 at refrigerator (Figs. 8 and 9). Similar trends reported by Effat (2000); Mehanna *et al.* (2003); Kebary, *et al.* (2004) and Kebary, *et al.* (2009).

Table 1: Sensory evaluation of white cheese manufactured with different salt concentrations during storage period.

salt conc. %	Ripening periods (days)	Room (20^0C)				Refrigerator(4^0C)			
		F	B &T	A	Total	F	B &T	A	Total
		60	30	10	100	60	30	10	100
(T1) 0 salt	Fresh	44	19	7	70	43	18	7	68
	30	45	22	7	74	45	22	8	75
	60	49	24	8	81	49	23	8	80
(T2) 3%	Fresh	44	18	7	69	43	23	7	73
	30	48	24	8	80	48	25	7	80
	60	51	25	8	84	50	25	8	83
(T3) 6%	Fresh	50	20	7	77	49	22	8	79
	30	52	22	8	82	51	24	8	83
	60	53	26	8	87	51	26	9	84
(T4) 10%	Fresh	47	20	7	74	48	22	7	77
	30	51	22	7	80	49	24	7	80
	60	52	23	8	83	53	25	8	86

F= Flavor, B & T =Body and texture, A= appearance

Fermented milk production using L paracasei 441 produced by batch and continuous biofilm reactor:

Changes in the viable counts of strain Lactobacillus paracasei 441 during storage period:

During storage, a slight increase in *L. paracasei* 441 number occurred in treatment T2 comparing with treatment T3 during 15 days of storage, the cells increased by an average of half of logarithmic cycle at (4°C±1).Depending on the source of cell production; batch or continuous biofilm cultures, we could notice that the viability and stability are higher in treatments with cells came from biofilm more than cells that came from batch by at least half log Fig. (10).

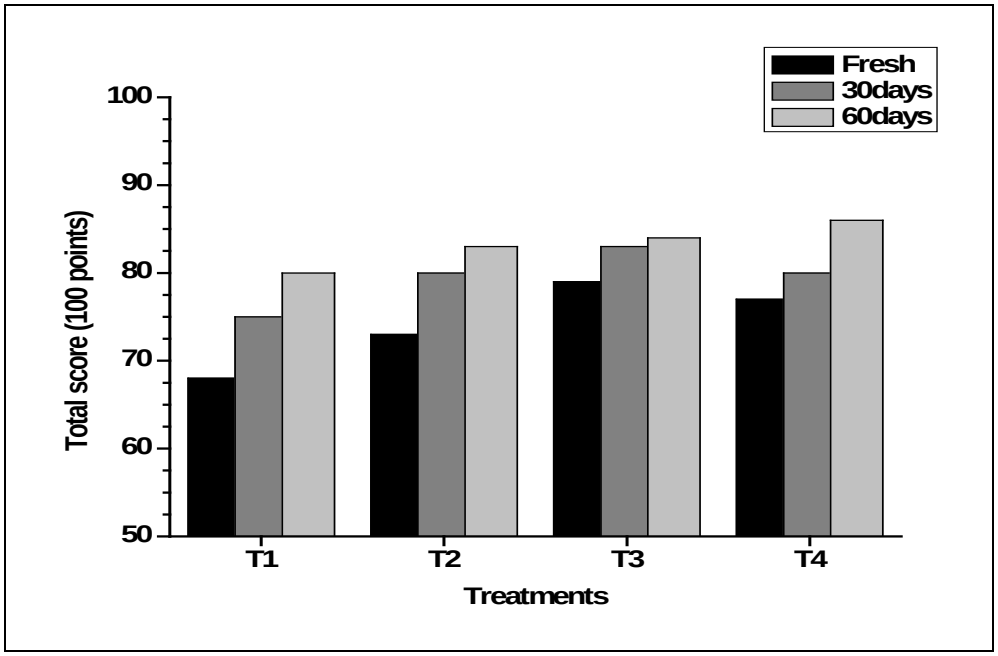

Fig. 8: Organoleptic properties of white cheese treatments made with different concentrations of salt and two sources of *L. paracasei* 441 strain during storage at refrigerator temperature (4^{o}C±1).

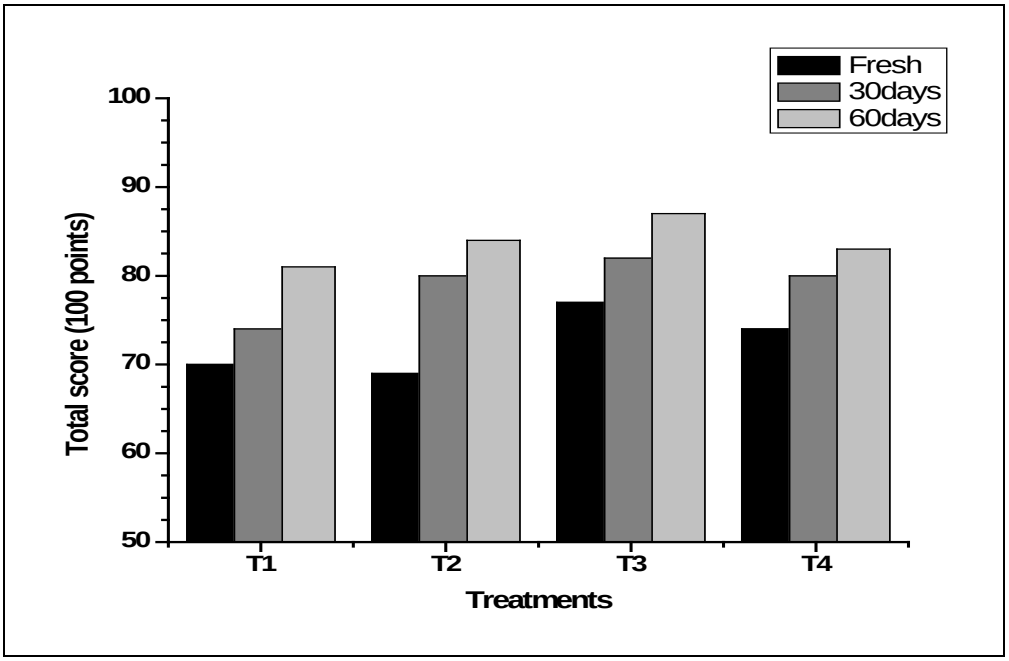

Fig. 9: Organoleptic properties of cheese treatments made with different concentrations of salt and two sources of *L. paracasei* 441 strain during storage at room temperature (20^{o}C±1).

In connection of these results similar pattern of tolerant effects of cells under acidic condition produced by biofilm was reported by Bruno Barcena *et al.* (1998); Bruno Barcena *et al.* (1999) and Bruno Barcena *et al.* (2001). In this respect, Cheng *et al.* (2010) and Dagher *et al.* (2010) reported that immobilized cells have advantages over free cells, such as protection from toxic substances, increased plasmid stability and increased catalytic activity.

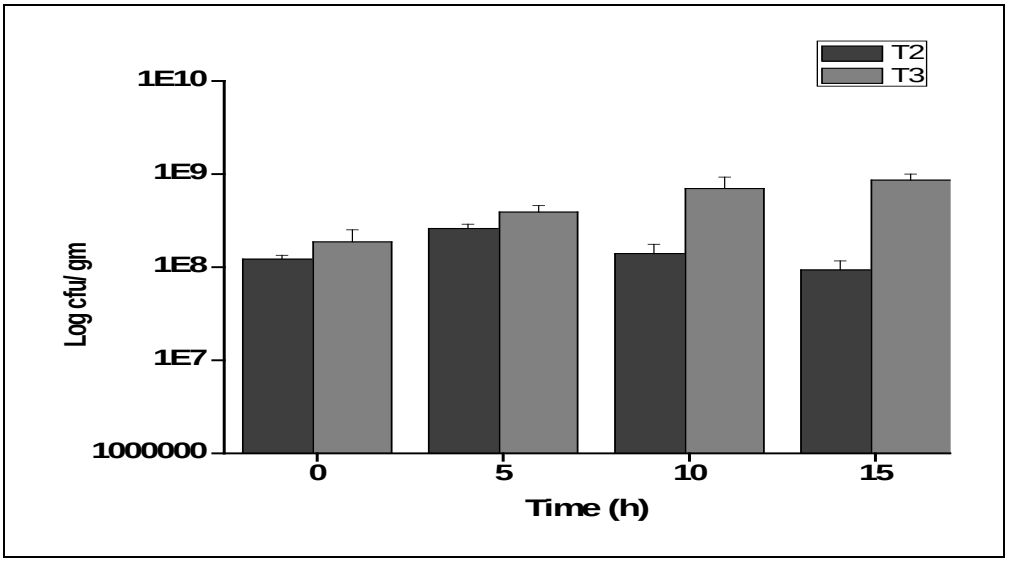

Fig. 10: Change in the numbers of viable *Lactobacillus paracasei* 441 of the set fermented milk made with two source of *L. paracasei* 441 during storage at refrigerator temperature (4°C±1). Error bars indicate the Standard error (SE) of the mean of triplicates.

Change in pH and titratable acidity:

Changes of the pH values of set fermented milk manufactured with added *Lactobacillus paracasei* 441 during storage at 4°C ±1 for 15 days are shown in Fig. (11). It could be noticed that pH values of fermented milk, were 4.79 when fresh and 4.55 after 15 days storage period respectively in control but with adding the new strain *Lactobacillus paracasei* 441 they were 4.74 and 4.73 for treatment T2 and treatment T3 when fresh respectively and were 4.5 and 4.44 after storage 15 days respectively, *i.e.* the pH values slightly decreased throughout the storage period at 4°C ±1.

Figure (12) illustrated the effect of *Lactobacillus paracasei* 441on the acidity of set fermented milk. The results clearly indicate that the acidity of treatments (T2 and T3) were 0.73 and 0.75 respectively when fresh comparing to control (T1) which was 0.74 and increased to 0.9 , 0.94 and 0.89 for treatments (T2 and T3 and control) respectively after storage period of 15 days. Treatments were higher than the control during storage till the end of the period (15days). This could be due to that activity of *Lactobacillus paracasei* 441 in the product. On the other hand the values of acidity of cell came from biofilm (T3) is higher than that cells came from batch culture (T2), which indicate that the cells are active and more tolerant to acidity than cells produced by batch.

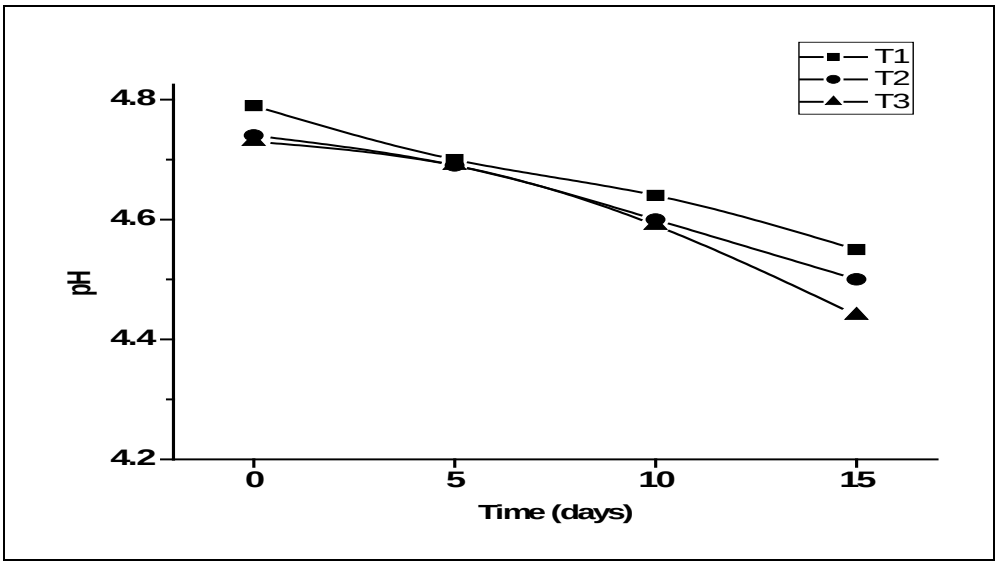

Fig. 11: Changes in pH values of set fermented milk made with two source of *L. paracasei* 441 during storage at refrigerator temperature (4°C±1).

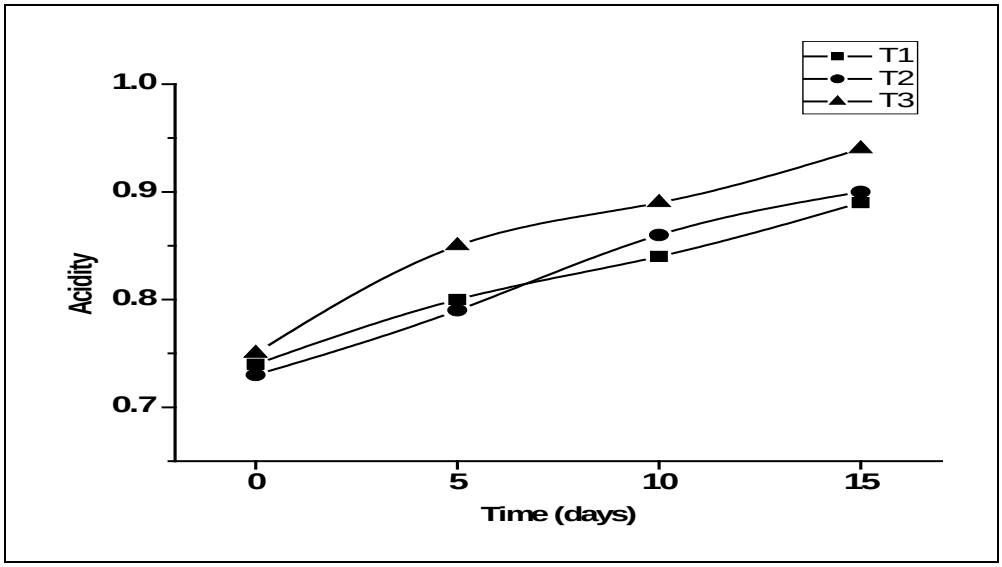

Fig. 12: Changes in Acidity of set fermented milk made with two source of *L. paracasei* 441 during storage at refrigerator temperature (4°C±1).

Rheological properties of the set fermented milk:

Syneresis:

Changes of Syneresis of set fermented milk (SFM) are shown in Fig. (13). Results illustrated that the syneresis of treatments with *Lactobacillus paracasei* 441 (T2 and T3) were lower than the control (T1) when fresh and during storage at 4°C±1, till the end of 15 days. These results indicate that effect of production polysaccharides by *Lactobacillus paracasei* 441 in (T2 and T3). Similar results were reported by Ruas-Madiedo *et al.* (2002a and 2002b).

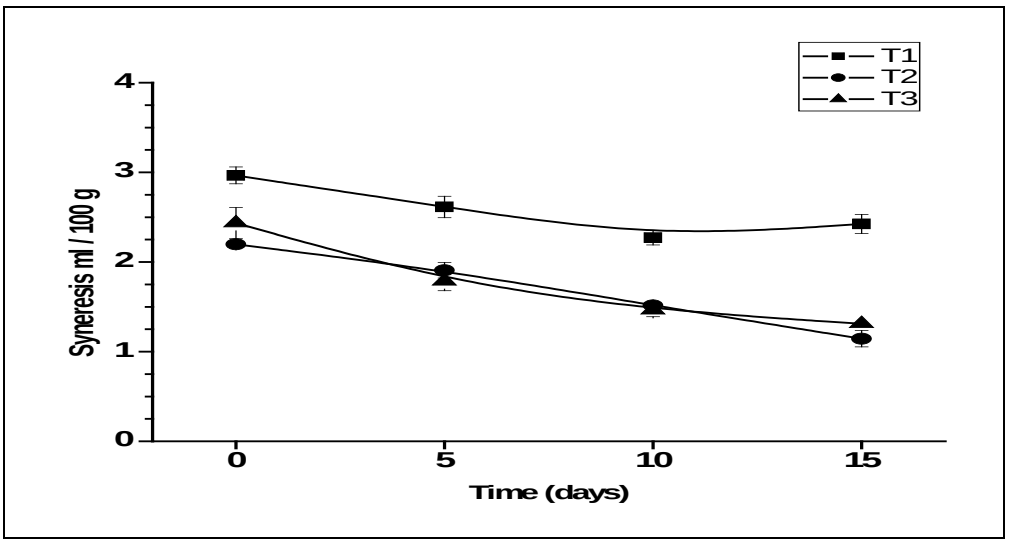

Fig. 13: Changes of syneresis in set fermented milk made with two source of *L. paracasei* 441 during storage at refrigerator temperature (4°C±1). Error bars indicate the Standard error (SE) of the mean of triplicates.

Viscosity:

Is shown in Fig. (14). The control treatment (T1) had less viscosity than (SFM) produced by strain *Lactobacillus paracasei* 441. Differences in the viscosity rate were observed throughout storage period and the values of viscosity of treatments when fresh were 29.66, 31.975 and 31.835 for treatment T1, T2 and T3 respectively and were 35.00, 53.95 and 52.11 respectively after 15 days storage period. There were significant differences between control and the two treatments manufactured with strain *Lactobacillus paracasei* 441. On

the other hand, there were no significant differences between T2 and T3 which cells produced by batch and continuous biofilm culture respectively.

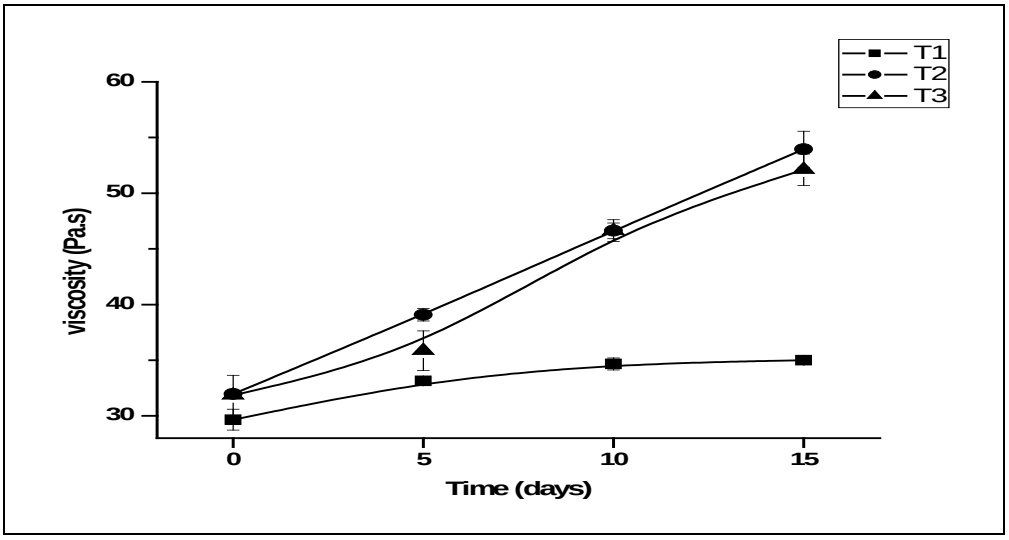

Fig. 14: Changes of viscosity in set fermented milk made with two source of *L. paracasei* 441 during storage at refrigerator temperature (4°C±1). Error bars indicate the Standard error (SE) of the mean of triplicates.

Sensory evaluation of the set fermented milk:

In conclusion the acceptability of previous set fermented milk treatments could be ranked as follows T2>T3>T1 when storage at refrigerator for 15 days. Similar trends reported by Ruas-Madiedo *et al.* (2002a and 2002b) who suggested that polysaccharide improve the texture and keep the flavor of yoghurt. The set fermented milk manufactured with strain *L. paracasei* 441 produced from batch culture had gained the highest total scores compared to the control manufactured without addition the strain. This may be due to the effect of production of polysaccharides and improving the Rheological properties of fermented milk.

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