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EFFECT OF ADDING SOME LACTIC ACID BACTERIA ON THE ACCELERATION RIPENING OF RAS CHEESE SLURRY

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ABSTRACT

Three strains of lactic acid bacteria were used as adjunct bacteria with the normal starter of Ras cheese. The strains were grown in Ras cheese slurry for 15 days. The influence of each strain was assessed on the basis of the biomass growth, rate of autolysis, rate of both proteolysis and lipolysis. The results indicated that the addition of *Lactococcus. Lactis sub.lactis* gave an excessive stimulation effect on biomass formation and autolysis for either *streptococci* or *lactobacilli.* The biochemical changes in the slurry due to the type of the adjunct bacteria revealed to the disadvantages of using *Lactobacillus casei* as it showed lower pH value, higher lipolysis and lower proteolytic activity. This effect might be related to the low activity for biomass formation as well as to the lower lysis ability. The results revealed to the superiority of *Lactococcus lactis sub. lactis* for biomass production, autolysis and proteolysis. On the other hand, the strain of *Streptococcus thermophilus* showed an advantage effect on growth and lysis of *Streptococci* only.
Keywords: Ras cheese, cheese slurry, proteolysis, lipolysis.

INTRODUCTION

During cheese ripening, the cheese undergo numerous biochemical changes, which lead to development of appropriate flavour and aroma. This is a result of a complex process which involves many ripening agents. The principal ripening agents involved in cheese ripening are: Indigenous milk enzymes, starter bacteria, coagulant, secondary starter and the microflora. Assessment of the contribution of the principal ripening agents is an expensive and time consuming process due to the long periods (6 – 12 month) required for full flavour development (El-Soda and Pandian, 1991 and Fox, 1993).

Microbial consortia are able to develope valuable properties which are often the result of the activities of a group of microorganisms rather than a single one. This is the case for cheese ripening, which is made possible by a complex ecosystem in which bacteria and rather microorganisms are involved. The use of several bacterial strains as adjunct for cheese ripening is common, as it improves flavour and accelerate ripening; so the selection of appropriate adjunct is a key for successful cheese production. As the evaluation of each bacterial strain individually for its impact in cheese quality would be costly and time consuming, the use of cheese curd slurry system offers a solution to this problem. Therefore, slurries may be used to screen organisms for their potential to directly predict cheese ripening (Fox et al., 1996).

So, the present study extends the applicability of the slurry systems for investigating changes brought about by the use of different adjunct bacteria within Ras cheese variety. Three strains of bacteria, namely:

Lactbacillus casei (EMCC 11093), *Lactococcus lactis sub lactis* (EMCC 11552) and *Streptococcus thermophilus* S3588 (Encapsulated strain) were co-cultured with the normal Ras cheese starter. So far, possible interactions between cheese bacteria in cheese ecosystem have been assessed in terms of growth and aroma compound synthesis in cheese based medium.

MATERIALS AND METHODS

Materials:

Milk samples: Whole fresh buffalo's milk was obtained from Animal Production Research Institute, Min. of Agric. Giza.

Microbial strains: Ras cheese starter consisted of *Lactobacillus delbrueckii* sub *bulgaricus* (EMCC 11102) and *Streptococcus thermophilus* (EMCC 11044), adjunct starter consisted of *Lactobacillus casei* (EMCC 11093), *Lactococcus lactis* sub. *lactis* (EMCC 11552) were obtained from MIRCEN and *Streptococcus thermophilus* (S3855**)** were obtained from Cairo Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University.

Strains preparation for the slurry: Preparation of adjunct cultures was carried out according to El-Shafei (1994). Late logarithmic phase of *Lactobacillus casei*, *Lactococcus lactis* sub. *lactis* and *Str. thermophilus* (S3855) (48 h of incubation) were collected from the growth medium by centrifugation at 4500 rpm for 20 min at 4°C and washed three times with 0.01 M phosphate buffer (pH 7.0). The obtained pellets were added to 50 ml sterilized skim milk and incubated at 30 ºC for 24 h prior to use in cheese milk for manufacture of Ras cheese curd.

Preparation of Ras cheese slurry: Ras cheese slurry was prepared from buffalo milk as follows: Milk was heated to 70 ºC for 30 sec, then rapidly cooled to 35ºC and cultured with normal Ras cheese starter (2%), then, divided into four portions. Portion I was served as control, portion II was treated to give slurry supplemented with *Lactobacillus casei* (EMCC 11093), signed as treatment 1(T1). Portion III was treated to give slurry supplemented with *Lactococcus lactis* sub. *lactis* (EMCC 11552) , signed as treatment 2(T2),while portion IV was treated to give slurry supplemented with *Streptococcus thermophilus* (S3855), signed as treatment 3(T3). Each portion was manufactured into Ras cheese curd to use in slurry preparation as described by Abd-El-Tawab (1963).

Methods: The cheese slurry was analysed for pH, titratable acidity, moisture content, salt, total nitrogen, water-soluble nitrogen (WSN) and non-protein nitrogen (NPN) using methods described by AOAC (1990). Free amino acids(FAA)content was estimated according to the method of Folkertsma and Fox (1992).

Microbiological analysis for cheese and cheese slurry were carried out in duplicate at each time of sampling. *Lactobacilli* spp counted on MRS agar after incubation for 48 h at 32 ºC. *Streptococci* and *Lactococci* were also counted on M17 agar after incubation for 48 h at 32 ºC.

RESULTS AND DISCUSSION

Effect of adjunct bacteria on the growth and autolysis properties of bacteria in the slurry:

The effect of addition different adjunct strains on the growth and autolysis properties of bacteria in the slurry as enumerated onto M17 for *Streptococci* and MRS for *Lactobacilli* were shown in Figures (1 and 2). The results indicated an increase in the bacterial count as observed through the early incubation periods and it was at maximum at 3 days, then it declined continuously up to the end of the incubation periods. The maximum bacterial count of *streptococci* ranged from 5.86×10^9 to 2.21×10^{10} (cfu/g) after 3 days. The corresponding population for *Lactobacilli* was: 3.23×10^9 to 9.40×10^{10} (cfu/g), on the same days.

Similar behaviour for the changes in the bacterial counts was also reported by Madkor *et al.* (1999) reported that the viable starter of Lactic acid bacteria (LAB) increased one day after manufacture (10^8 – 10^9 cfu/g cheese) counts was at least 4-5 log cycle higher than the others .

The effect of the adjunct bacterial cells on the biomass formation during slurry preparation is differed among strains. The results indicated that the addition of *Lactococcus. lactis* sub. *lactis* as adjunct bacteria gave an excessive stimulation effect on biomass formation either for *Streptococci or Lactobacilli* compared with *Lactobacillus casei* which gave a negative effect on biomass formation. The strain of *Streptococcus thermophilus* S3855 showed a selective effect, as it increases the biomass of *Streptococci*, only. This effect of the adjuncts bacteria on biomass formation was more obvious when the bacterial biomass of the treatments was high when compared with that of the control. The maximum bacterial count of *Streptococci* for slurries T1, T2 and T3 were 1.158, 2.849 and 3.771 fold than the control. The corresponding values for the *Lactobacilli* were: 1.303, 16.578 and 0.530 fold than the control, respectively. These variations in the bacterial counts may be reflected the relationship between the starter culture and adjunct strains, which could stimulate or inhibit the growth of each others. These results proven that the role of bacterial strains not only contributes to acid production but will also make a clear contribution to the biomass of LAB in young curd. It is also, supposed that the counts or biomass formation is a criterion for screening between adjunct bacterial strains.

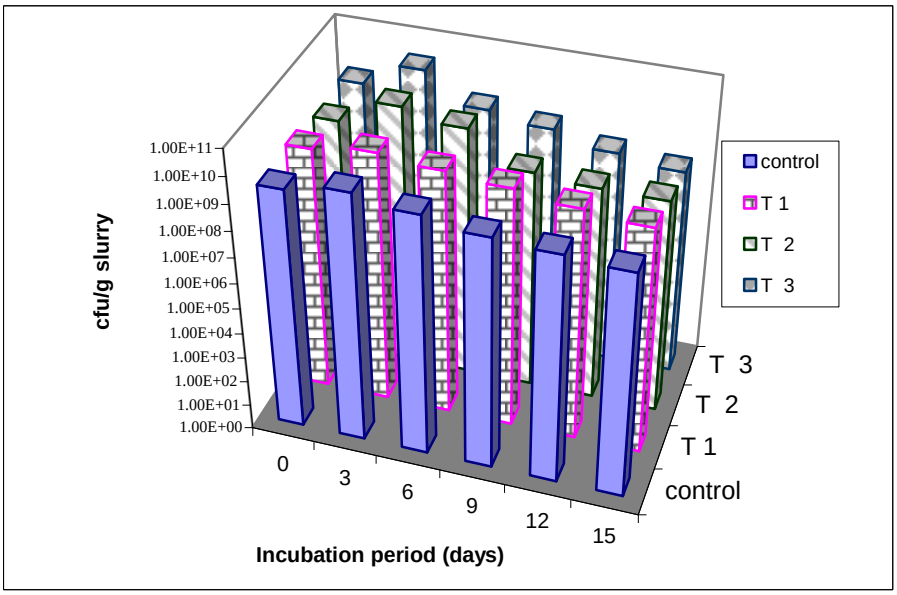

Figure (1): Changes in *Streptococci* count during incubation periods of Ras cheese slurries.

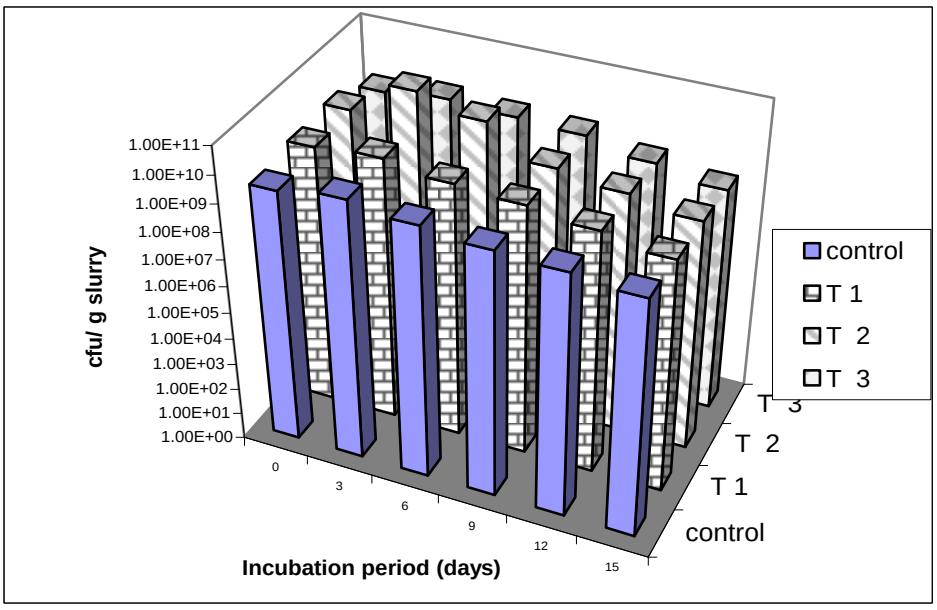

Figure (2): Changes in *Lactobacilli* count during incubation periods of Ras cheese slurries.

The trend of decreasing the counts of the biomass in the slurries after 3 days up to the end of the incubation periods was shown in Table 1 and Figures 1,2. Robert *et al* (1995) found that count in slurries genera increased 100-to 1000-fold during ripening. Also, Dias and Weimer (1999) found that starter culture population ranged from 2.5×10^8 to10×10^8 (cfu/g) at zero time and decreased to 6×10^5 – 1.9×10^6 (cfu/g) at 14 days. This behaviour of the biomass decrease after the maximum increase was defined as autolysis or lysis. The rate of lysis was evaluated by the rate of the decrease in the counts of viable cells during the incubation periods. The percentages of lysed cells of *Streptococci* and *Lactobacilli* were 88.45 and 85.20%(control) ; 98.270 and 99.458% (T1); 87.31 and 90.79%(T2) and 99.34% and 91.03% (T3). Bacerial biomass has to be taken into account during the selection of industrial strains of lactic acid bacteria.

Biochemical changes during the ripening periods of the slurry:

Changes in pH value and acidity:

The changes in the pH value and acidity during the ripening periods of the slurry as affected by the strains of the adjunct bacterial cultures are shown in Table (1). The results showed that very fast increase in the acidity and a sharp decrease in the pH value during the first six days of the incubation periods then these changes slowed down up to the end of incubation periods. The influence of the adjunct strains on the changes in the pH value and acidity during the incubation periods were almost the same for all slurries except in T1 which showed a more decrease in the pH value and more increase in acidity than the others. At the end (15 days) of the incubation periods, the pH value and acidity of the slurries were 3.64 and 2.76 % for T_1, compared with 4.10 to 4.17 and 2.75 to 2.70%, for other treatments. The differences between treatments may be related to the differences in the activity of strains, which might be affected by the manufacturing and the autolysis conditions during the ripening periods of the slurry. The profile of the changes in pH value and acidity of T_1 may be related to the resistance of microbial strains against cheese manufacture and slurry conditions, thereby more fermentation for lactose and higher production of acidity were observed. These results agreed with Para *et al.,* (2000) whom found that the pH value of the slurries at different incubation periods was 5.4 in all cases. After the first 2 and 8 days, pH value was dropped significantly in the experimental slurries to an average of 5.0 and 4.8, respectively, as a result of microbial growth. The pH value was not change after 8 days of incubation. The pH value of the control at the same periods was 5.1.

Table (1): Changes in the pH value and Titratable acidity (TA) in Ras cheese slurry.

Incubation periods / days	Slurry sample							
	Control		T 1		T2		T 3	
	pH	T.A%	pH	T.A%	pH	T.A%	pH	T.A%
0	6.55	0.30	5.74	0.45	6.65	0.25	6.72	0.28
3	4.75	1.65	4.31	2.00	4.82	1.50	4.84	1.55
6	4.40	2.33	3.83	2.63	4.37	2.03	4.34	2.25
9	4.25	2.40	3.65	2.75	4.17	2.30	4.22	2.30
12	4.21	2.45	3.65	2.76	4.17	2.75	4.19	2.60
15	4.20	2.50	3.64	2.76	4.10	2.75	4.17	2.70

T1: with Lactobacillus casei T2: with Lactococcus lactis sub lactis T3 : with Streptococcus thermophilus

Extent of proteolysis:

Proteolysis was estimated by the determination of the water-soluble nitrogen (WSN), non-protein nitrogen (NPN) , and free amino acid nitrogen (FAAN). The NPN is widely used, in combination with WSN to describe the ratio of proteolysis.

Water-soluble nitrogen (WSN):

The WSN, expressed as WSN/TN, of the different slurries compared with the control is shown in Figure (3). WSN indicated more information about proteinase activity and characterizes ripening into the wide degradation of casein to water soluble fragments.

The results indicated an increase in WSN/TN in the slurry with the increase in the incubation periods. At the end of the incubation periods this increase was about 2-fold for control and T_1 while it was 3-fold for T_2 and T_3. The WSN/TN content at the beginning of the incubation time were: 8.93, 8.93, 4.69 and 5.03 % for the control, T_1, T_2 and T_3, respectively. While the corresponding values after 15 days of the incubation were: 15.11, 13.73, 13.87 and 14.17, respectively. These results indicated that, after 15-day of ripening, a negligible difference in WSN/TN was observed between slurries and the fast increase in treatments T_2 and T_3 was coincided with the previous results and had higher biomass counts and higher autolytic activity than control or T_1. The activated enzymes of T_2 and T_3 seemed to be the main factor responsible for the fast proteolysis of the slurries. The *Lactobacillus. casei* (T_1) revealed little proteolysis. This may be due to the resistance of this strain to autolytic process as reported before and as mentioned by Madkor *et al* (1999) . The increased in soluble nitrogen have been reported by Visser, (1977)and Exterkat and Alting (1995).

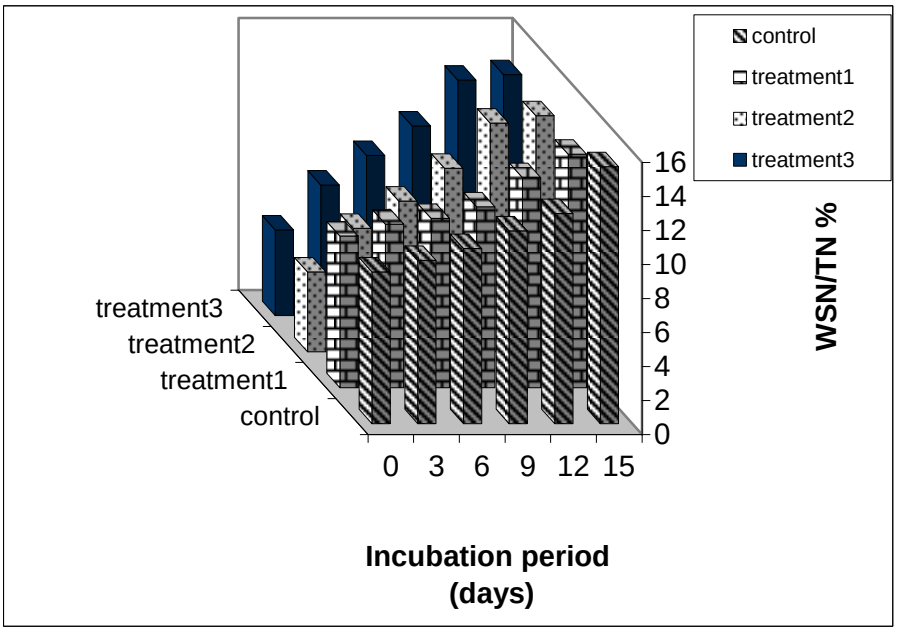

Figure (3): Water soluble nitrogen as percentage of total nitrogen (WSN/TN%)during incubation periods of slurries.

Non protein nitrogen (NPN):

The NPN gives more details on the peptidase activity on WSN to smaller fragments, it gave more details on the peptidase activity, therefore ripening into wide degradation of WSN to smaller fragments (Puhan and Steffen 1967). Results in Figure (4) showed that the trend of NPN between treatments during the incubation periods was the same as that for WSN. This confirms the previous results that T_2 and T_3 have more proteinase and peptidase system than that of the control or *Lactobacillus. casei* (T_1). Increasing in the NPN expressed as NPN /WSN, may be related to the increase in the degradation of WSN fraction during ripening periods which is an indicator to the high peptidase activity. The magnitude of the change in the NPN /WSN during the incubation periods of the slurries were 61.54 and 52.27(control); 61.51 and 57.49(T1); 45.50 and 53.83 (T2); 43.02 and 53.35(T3) at the beginning and after 15 day of incubation periods, respectively. This behavior is coincided with the rate of bacterial lysis during the incubation periods as shown before.

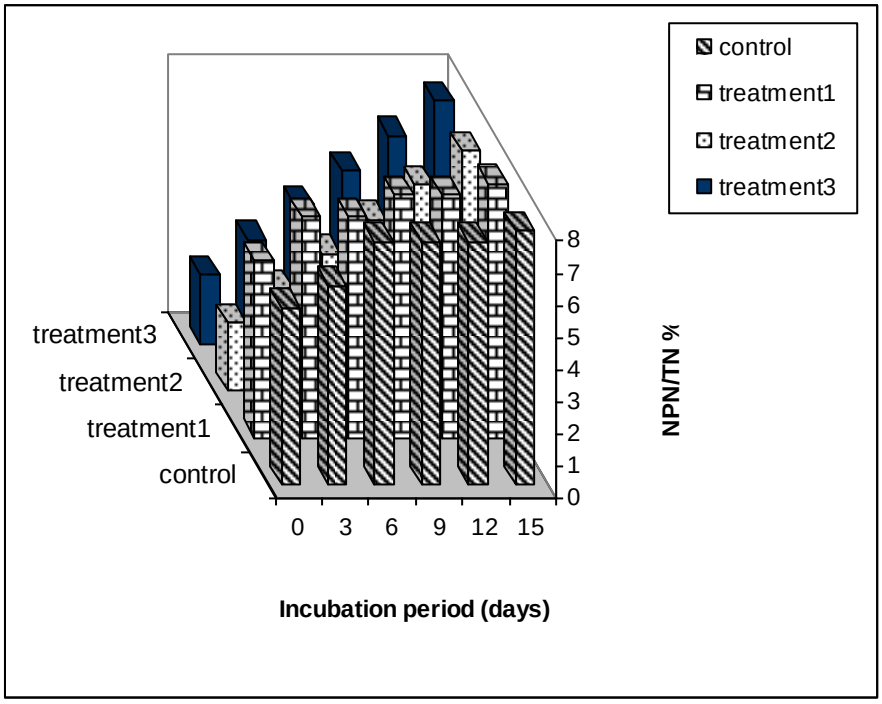

Figure (4): Non protein nitrogen as percentage of total nitrogen (NPN/TN %) during incubation periods of slurries.

Free amino acid nitrogen (FAAN):

Analysis of WSN extract of the cheese slurries by the α-ninhydrine method (which is highly sensitive for free amino acids) are shown in Figure (5). The results indicated that the slurries prepared with adjunct bacterial strains contained considerably higher concentrations of free amino acids than the control. The differences observed between the control slurry and adjunct treated slurries appear to be attributed to the high activity of adjunct peptidases and to the high ability of the adjunct cultures to release their intercellular peptidases in cheese matrix (Johnson *et al*, 1995). This result is in agreement with Madkor *et al*, (1999). These results support the previous results that T_3 and T_4 are favoured as good slurry for cheese ripening acceleration.

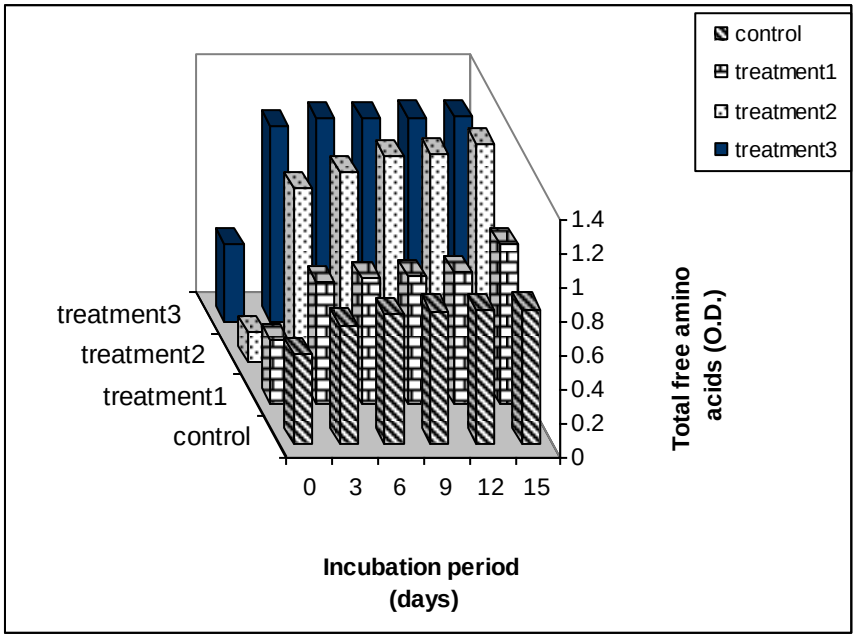

Figure (5): Total free amino acids (O.D.) at 507 nm during the incubation periods of slurries.

Extent of lipolysis:

As the extent of Lipolysis have less contribution in the flavour components of Ras and Cheddar cheese than the extent of proteolysis, it is very important for the choice between starter or adjunct strain on its extent of lipolytic ability. Results in Figure (6) mentioned that TVFA of the control and adjunct strain treatments. The results revealed an increase in TVFA in T_1 (which contain *Lactobacillus. casei*) compared with the control or T_2 andT_3. This result may be used to confirm the less importance of *Lactobacillus. casei* as an adjunct to cheese starter for cheese ripening acceleration.

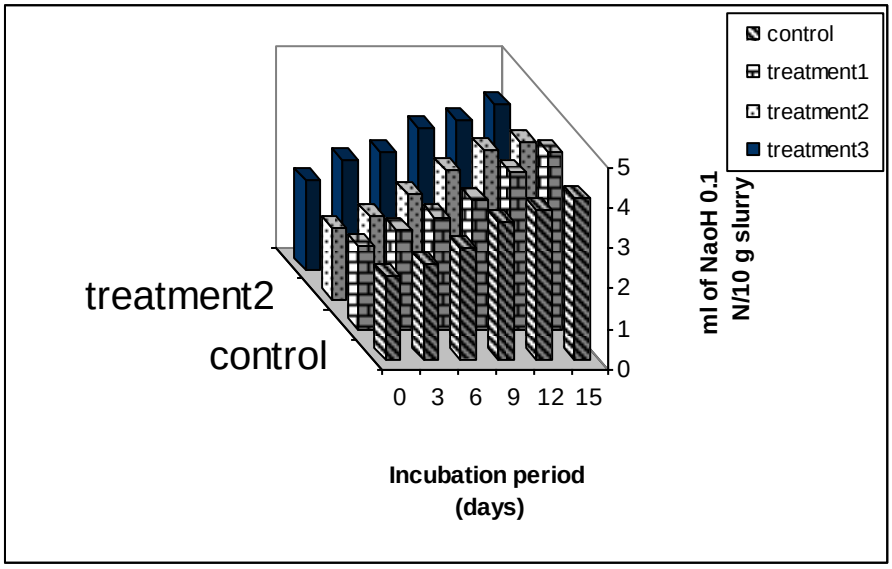

Figure (6): Change in total volatile fatty acid (TVFA) content of cheese slurries during incubation periods.

Conclusion

It could be concluded that the use of *Lactobacillus. casei*, *Lactococcus. lactis* sub *lactis* or *Streptococcus. thermophilus* S3855 as adjunct strains with the conventional starter of Ras cheese for ripening acceleration gave some discriminations between the strains. The counts of the biomass and lysis capacity as well as the rate of proteolysis and lipolysis are important criterions should be used to select between the adjunct bacterial strains.

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تأثيرإضافة بعض انواع من بكتيريا حمض اللاكتيك على اسراع تسوية معلق الجبن الراس

نادية محمد شاهين ، محمود حمزة السنيطي و نبيل عبدربه

قسم الالبان - المركز القومي للبحوث -القاهرة

في هذه الدراسة تم استخدام ثلاثة أنواع من بكتيريا حمض اللاكتيك بالاضافة الى البادئ الاساسي المستخدم في صناعة الجبن الراس ودراسة تأثير هذة الانواع على اسراع تسوية معلق الجبن الراس وذلك خلال ١٥ يوم . واوضحت النتائج على أن إضافة *Lactococcus lactis sub. lactis* مع البادئ الاساسي أدى الى اسراع تحلل البروتين والدهن فى معلق الجبن الراس الناتج عنه في المعلق المضاف له Streptococcus thermophilus أو Lactobacillus , casei L. casei - كما اظهرت التغيرات الكيميائية في المعلق الناتج والمستخدم في تصنيعه انخفاض في كل من درجة ال pH وتحلل البروتين مع زيادة في تحلل الدهن بالمقارنه بالانواع الأخرى .

كما أظهرت ايضا النتائج ان المعلق المستخدم في تصنيعه L. lactis sub lactis هو احسن بادئ استخدم في تصنيع المعلق مقارنة بالبادئات الاخرى. وعلى الجانب الأخر وجد ان استخدام بادئ S. thermophilus فى التصنيع كان له تأثير واضح على نمو البادئات عموما.