J. Food and Dairy Sci., Mansoura Univ., Vol. 9 (3): 121 - 126, 2018

The Combined Effect of Fortification of Media with Peptone and Control of Acidity on the Growth of Lactic Acid Bacteria

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ABSTRACT

The present work was carried out to investigate the combined effect of fortification of media with 1% peptone under control of culture acidity on the growth rate of *Lactococcus lactis* subsp*lactis , Lactococcus lactis* subsp *cremoris* and *Lactobacillus casei* in sterilized skim milk incubated at 34°C. The obtained results indicated that:*Lactococcus lactis* subsp *lactis* grow in sterilized skim milk resulted in coagulation of milk after 12th hour of incubation, and was reached plate count of 28x10^6 CFU/ml.When the same strain grown in sterilized milk supplemented with 1% peptone under control of culture acidity no coagulation was observed up to the end of incubation period after 28th hour at 34°C, and it reached a plate count of 70x10^6 CFU/ml at the end of the exponential phase after 12th hour of incubation Cessation of growth in the culture started the stationary phase after 24th hour of incubation. The effect of added 1% peptone to sterilized skim milk under control of culture acidity on the colony forming units of *Lactococcus lactis* subsp *cremoris*, indicated that control sample grown in sterilized skim milk reached count of 55x10^6 CFU/ml after 12th hour of incubation. The corresponding values for *Lactococcus lactis* subsp *cremoris* grow with medium supplementation and control of culture acidity reached a plate count of 20x10^7 CFU/ml after 24th hour of incubation.When *Lactobacillus casei* grow in sterilized skim milk incubated at 34°C with medium supplementation with 1% peptone under control of culture acidity, the population density was several times higher in culture supplemented with peptone under control of culture acidity reached a population density of 17x10^7 CFU/ml, while only 23x10^6 CFU/ml were recorded by the same strain being grown as a control after 24th hour of incubation.

Keywords: Lactic acid bacteria, titratable acidity, colony count, culture acidity control, Mrs Media

INTRODUCTION

Lactic acid bacteria are a group of gram- positive, non sporeforming, an aerobic or facultative aerobic cocci or rods, which produce lactic acid as one of the main fermentation products of the carbohydrates metabolism. Lactic acid bacteria play a critical role in food production and health maintenance (Quinto *et al.,* 2014).

During milk fermentation processes, lactic acid bacteria are exposed to various environmental stress conditions, such as temperature fluctuations, acidity, pH and decrease of available nutrients. Some of these conditions will often coincide.

Effects of some environmental parameters on growth kinetics of lactic acid bacteria might provide useful information concerning physiology and different reactions of the microorganism during growth under different conditions. Among these parameters pH, temperature, NaCl, water activity (a_w) and medium supplementation seems to be of great importance, Tanigughi *et al*. (1987) and Bernard-Bibal *et al*. (1988).

Several researchers have investigated the associate growth relationship between these parameters and *Streptococci* group, widely used as starters in dairy industry, Rhee and Rack (1980), Hassan and Richard (1990), Ismail (1990 a & b and 1991 a & b) and Ismail and Juillard (1991 a & b).

The optimum pH for growth of various strains of lactic acid bacteria have been determined by many investigators, Blickstad and Molin (1981) and Roy *et al.* (1986). However the extent to which the medium pH can influence the growth rates of individual species is less clear (Van de Guchte *et al.,* 2002)

The inability of lactic streptococci to synthesize many amino acids makes them dependent on an exogenous supply of amino acids and small peptides. Since fresh milk contains only a small amount of free amino acids and small peptides, these organisms are dependent on their proteoytic enzymes which hydrolyze casein (Lawrence *et al*., 1976).

Evidence presented by Ismail (1991a) suggested that an intensive growth of *Lactococcus lactis* subsp *lactis* was observed in autoclaved reconstituted milk and broth.

The fortification of milk with proteose peptone alone enriches the medium and supports the growth. He reported also that the cultivation of lactic acid bacteria in milk fortified with proteose peptone gave higher cell productivities as compared with the corresponding milk. Therefore, the aim of the present study was to elucidate the combined effect of supplementation of media with 1% peptone and control of culture acidity on growth kinetics of LAB in milk.

MATERIALS AND METHODS

Milk:

Buffalo milk used in this study was obtained from Faculty of Agriculture herd, morning milking. As soon as milk was arrived to the laboratory it was skimmed by using Alfa- Laval separator operated at 16000 rpm.

Skim milk was distributed into 1 L conical flasks; each flask contained 750 ml skim milk. Conical flasks containing skim milk were sterilized at 121°C/10 minutes (15 lph/inc^2)

Bacteria:

Lactic acid bacteria used in this study were:

Lactococcus lactis subsp *lactis*
Lactococcus lactis subsp *cremoris*
Lactobacillus casei

All starter cultures were obtained from the culture collection of Botany Department, Faculty of Science, Assiut University.

All organisms were routinely maintained in sterile litmus milk fortified with0.1% peptone and stored at5–7°C.

For the preparation of the inoculant, the procedure described by Hassan *et al.* (1989) was adopted. From each stored bacterial culture, a 1/10 dilutions were prepared in 500 ml conical flasks each one contained 250 ml sterilized skim milk incubated at 34°C.

The first non-coagulated flask in which the bacteria was expected to be in the exponential phase of growth was used for inoculating the experimental flasks inoculation was carried out by using certain volumes from the first non-coagulated flask to achieve about 105 CFU/ml in the experimental flasks.

Growth media:

MRS media was used in this study.

Bacterial analysis:

Enumeration of bacteria was carried out on MRS media. Petri dishes were incubated aerobically at 34°C for 48 hr. Colonies were determined by direct visual inspection.

Chemical analysis:

Determination of Developed Titratable Acidity (DTA):-

Developed titratable acidity was determined according to (A.O.A.C., 2000) by using sodium hydroxide N/9 and phenolphthalein as indicator.

Experimental:-

Each of the studied bacteria was inoculated into two of 1 L conical flask, each one contained 750 ml of sterilized skim milk. Inoculation was carried at about *x*105 CFU/ml at zero time. Conical flasks were immersed in water bath thermostatically controlled at34 ± 0.5°C.

The first flask was considered as control, while the second one was fortified with 1% peptone before sterilization. At each sampling time, calculated volumes of 0.1 N sodium hydroxide solution were added to adjust the acidity of culture as it was at zero time. Sampling was carried out at zero time and after each two hours intervals up to 28 hours of incubation at each sampling time 15 ml aliquots of each culture was aseptically withdrawn in 25 ml sterilized conical flask, 1 ml of the aliquots was aseptically withdrawn in a test tube containing 9 ml sterilized distilled water to give the first dilution 1/10 for the bacteriological analysis (10-1).

The dilution was shaked gently for 30 second, was used for the preparation for other dilutions 10-2, 10-3, 10-4, 10-5, 10–6.

RESULTS AND DISCUSSION

It is well known that shapes of all microorganism growth curves and the duration of each phase depend on several factors. However several explanation have been offered for the cessation of growth in the microbial cultures and starting the stationary phase of growth so tried in this study to investigate the effect of medium supplementation with 1% peptone under control of the culture acidity on the shape of the growth curves of three examined strains of LAB, and how these two factors can affect the duration of each phase. The obtained results are presented in Tables 1, 3 and 5.

Control samples growth in sterilized skim milk a steep, lag phase was found between 0 to 2nd hour in *Lactobacillus casei* and *Lactococcus lactis* subsp *cremoris*. In contrast, the duration of lag phase was from 2nd to 4th hour in *Lactococcus lactis* subsp *lactis*, reaching a colony count of $18x10^5$, $36x10^5$ and $12x10^5$ CFU/ml, respectively growth and acid production were in correspondence of each of the three variants grown in sterilized skim milk and throughout the incubation period.

Lactococcus lactis subsp *cremoris* and *Lactobacillus casei* grew at steady rate according to acid production and colony forming units were shown in figs 3, 4, 5 and 6. On the other hand, the rate of growth of *Lactococcus lactis* subsp *lactis* grow in sterilized skim milk was faster according to acid production and colony forming units, figures 1, 2.

Development of growth and acid production occurred by the examined strains grown in sterilized skim milk in the medium supplemented with 1% peptone under control of culture acidity at 34°C are shown in Tables 1, 2, 3, 4, 5 and 6.

Colony forming units assay clearly show the fundamental difference between *Lactococcus lactis* subsp *lactis*, *Lactococcus lactis* subsp *cremoris* and *Lactobacillus case* when *Lactococcus lactis* subsp *lactis* grow in sterilized skim milk, the sample was coagulated after 12 hour of incubation, and plate count of $28x10^6$ CFU/ml was detected .On the other hand, the same strain being grown in sterilized milk supplemented with 1% peptone under control of culture acidity, no coagulation was noticed up to incubation period of 28th hour at 34°C, and it reached a plate count of $70x10^6$ CFU/ml at the end of the exponential phase after 24th hour of incubation. Cessation of growth in the culture, and starting the stationary phase was shown in Tables 1 and 2 and Fingers 1 and 2.

Table 1. Rate of increase 0f colony count of *Lactococcus lactis* subsp *lactis* cultivated at 34°C in sterilized skim milk supplemented with 1% peptone and control of culture acidity (log CFU/ml).

Sampling time (hour)	Control		Supplemented with 1% peptone and control of culture acidity	
	CFU/ml	Log CFU/ml	CFU/ml	Log CFU/ml
0	90×10^4	5.95	130×10^4	6.11
2	104×10^4	6.01	140×10^4	6.14
4	12×10^5	6.07	155×10^4	6.19
6	297×10^4	6.45	41×10^5	6.61
8	80×10^5	6.90	19×10^7	7.27
10	16×10^6	7.20	41×10^6	7.61
12	28×10^6	7.45	70×10^6	7.84
24	70×10^6	7.84*	15×10^7	8.17
26	73×10^6	7.86	18×10^7	8.25
28	80×10^6	7.90	20×10^7	8.30

***Coagulated**

Table 2. Rate of increase in titratable acidity (%) during growth of *Lactococcus lactis* subsp *lactis* cultivatedat 34°C in sterilized skim milk supplemented with 1 % peptone and control of culture acidity.

Sampling time (hour)	Control	Difference in acidity	Supplemented with 1% peptone and control of culture acidity	Difference in acidity
0	0.20	0.00	0.20	0.00
2	0.22	0.02	0.22	0.02
4	0.23	0.03	0.20	0.00
6	0.24	0.04	0.21	0.01
8	0.25	0.05	0.20	0.00
10	0.26	0.06	0.22	0.02
12	0.27	0.07	0.20	0.00
24	0.42*	0.22	0.53	0.33
26	0.43	0.23	0.20	0.00
28	0.44	0.22	0.20	0.00

***Coagulated**

These results are in good agreement with those obtained by Hindle andWheelock (1970), under show a direct relationship exist between the population density and the availability of utilizable nitrogen's compounds apparently limits the extent of growth in milk. It was therefore to be expected that growth in supplementation of medium and control of culture acidity would reach higher population densities in milk supplemented with peptone, but to certain limit.

The effect of added 1% peptone to sterilized skim milk together with the control of culture acidity on colony forming units of *Lactococcus lactis* subsp *cremoris* are shown in Tables 3 and 4 and figures 3, 4.

Control sample grow in sterilized skim milk reached a plate count of 55x106 CFU/ml after 12 hour of incubation. The corresponding values for *Lactococcus lactis* subsp *cremoris* grown with medium supplementation under peptone and control of culture acidity reached a plate count of $90x10^6$ CFU/ml after 12 hour of incubation such findings agreed with those of Talaat and Allen (1971).

The large number of cells developed by *Lactococcus lactis* subsp *cremoris* in milk supplemented with peptone and control of culture acidity suggested that it may reach a higher population density than the same strain grown in sterilized skim milk, and would, therefore, be useful in mass culturing of *Lactococcus lactis* subsp cremoris. These results are in consistence with those observed by Mckay and Baldwin (1975), however Heap and Richardson (1985) found that higher product yield was obtained in recombined milk containing 0.1% yeast extract when *Streptococcus cremoris* was used.

When *Lactobacillus casei* grow at 34°C in sterilized skim milk in the absence of 1% peptone and of the controlled of culture acidity, and with fortification of the media with 1% peptone of the controlled culture acidity, the population density was several times higher in culture supplemented with peptone and control of culture acidity, reached a population density of $90x10^6$ CFU/ml, compared with $11x10^6$ CFU/ml by the same strain being grown as a control. This medium proved to be superior for lactic acid production Figures 5 and 6 these results agreed with those reported by Ismail (1991 b), Hindle and Wheelock(1970), and Thandon and Ganguli (1972).

As milk contains only small amounts of amino acids and small peptides, casseolytic activity is required for rapid growth and acid production when *Lactic streptococci* are grown in milk, so supplementation with peptone can help to obtain their needs of nitrogenous compounds, Speck (1962).

Fortification of milk with peptone and of the controlled acidity yielded an excellent growth for all studied strains as maximum levels of colony forming units was obtained.

Although it is customary in the dairy industry to measure culture activity either by growth or by acid production, these two indices are not always in correspondence, and various degrees of differences of cell growth from acid production are evident from colony forming units, Figures 1, 3 and 5 developed titratable acidity Figures 2, 4 and 6 in every case, rate of increase in DTA was higher than that of CFU. Which indicates efficient acid production and less cell growth.

Thus, sufficient cell mass production in a supplemented medium is possible and this may allow exclusive use in the dairy industry.

The effect of adding 1% peptone and control of culture acidity to the three bacteria strains was greater on *Lactococcus lactis subsp cremoris* compared to the other two strains. The lag phase wasn't observed clearly in case of *Lactococcus lactis subsp cremoris* unlike the other two cultures. This leads to reduce the lag phase and improve the productivity of cells to the degree that the number of cells became $24x10^8$ CFU/ml at 28th hour; this is the highest value noticed in the three cultures.

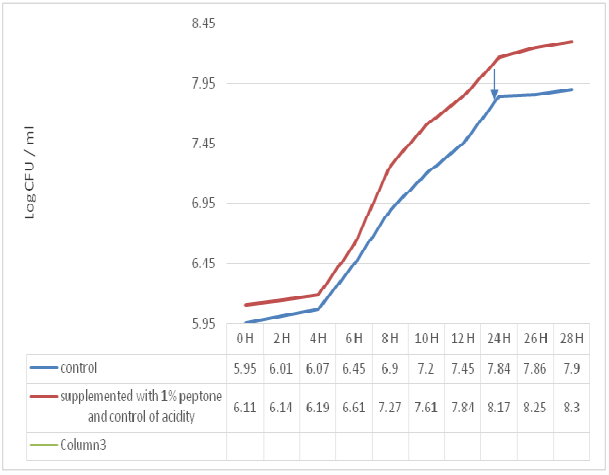

Fig. 1. Rate of increase 0f colony count of *Lactococcus lactis* subsp *lactis* cultivated at 34°C in sterilized skim milk supplemented with 1% peptone and control of culture acidity (log CFU/ml).

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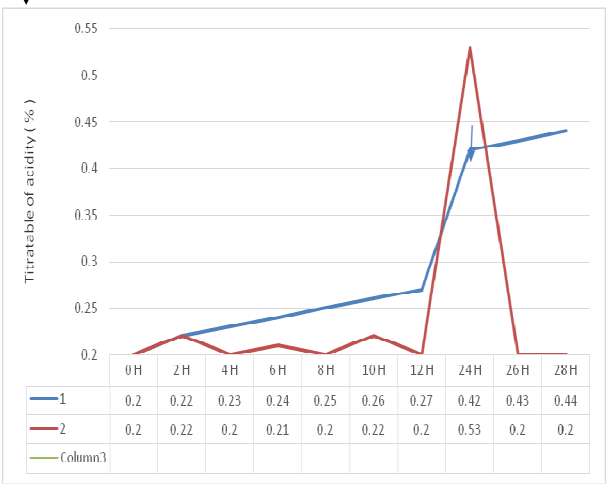

Fig. 2. Rate of increase in titratable acidity (%) during growth of *Lactococcus lactis* subsp *lactis* cultivatedat 34°C in sterilized skim milk supplemented with 1 % peptone and control of culture acidity.

(1) rate of increase in titratable acidity (%) during growth of *Lactococcus lactis* subsp *lactis* cultivated in sterilized skim milk at 34°C (control) .

(2) rate of increase in titratable acidity (%) during growth of *Lactococcus lactis* subsp *lactis* cultivated in sterilized skim milk supplemented with 1 % peptone and control of acidity at 34°C.

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Table 3. Rate of increase of colony count of *Lactococcus lactis* subsp*cremoris* cultivated at 34°C in sterilized skim milk supplemented with 1% peptone and control of culture acidity (log CFU/ml).

Sampling time (hour)	Control		Supplemented with 1% peptone and control of culture acidity	
	CFU/ml	Log CFU/ml	CFU/ ml	Log CFU/ ml
0	278 x 10^4	6.44	30 x 10^5	6.47
2	36 x 10^5	6.55	40 x 10^5	6.60
4	50 x 10^5	6.69	54 x 10^5	6.73
6	70 x 10^5	6.84	14 x 10^6	7.14
8	90 x 10^5	6.95	32 x 10^6	7.50
10	175 x 10^5	7.24	55 x 10^6	7.74
12	55 x 10^6	7.74	90 x 10^6	7.95
24	85 x 10^6	7.92*	20 x 10^7	8.30
26	12 x 10^7	8.07	90 x 10^7	8.95
28	10 x 10^7	8.00	24 x 10^8	9.38

*Coagulated

Table 4. Rate of increase in titratable acidity (%) during growth of *Lactococcus lactis* supsp *cremoris* cultivated at 34°C.in sterilized skim milk supplemented with 1 % peptone and control of culture acidity

Sampling time (hour)	Control	Difference in acidity	Supplemented with 1% peptone and control of culture acidity	Difference in acidity
0	0.20	0.00	0.20	0.00
2	0.22	0.02	0.22	0.02
4	0.23	0.03	0.20	0.00
6	0.25	0.05	0.21	0.01
8	0.26	0.06	0.20	0.00
10	0.27	0.07	0.23	0.03
12	0.28	0.08	0.20	0.00
24	0.42*	0.22	0.60	0.40
26	0.46	0.26	0.20	0.00
28	0.46	0.26	0.20	0.00

*Coagulated.

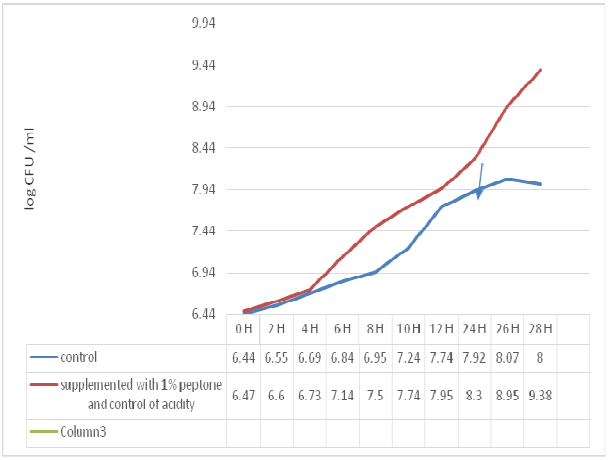

Fig. 3. Rate of increase of colony count of *Lactococcus lactis* subsp *cremoris* cultivated at 34°C in sterilized skim milksupplemented with 1% peptone and control of culture acidity (log CFU/ml).

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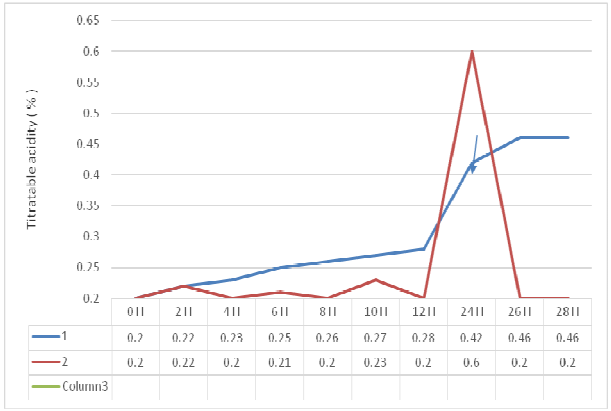

Fig. 4. Rate of increase in titratable acidity (%) during growth of *Lactococcus lactis* subsp *cremoris* cultivated at 34° in sterilized skim milk supplemented with 1% peptone and control of culture acidity .

(1) rate of increase in titratable acidity (%) during growth of *Lactococcus lactis* subsp *cremoris* cultivated in sterilized skim milk at 34°C (control)

(2) rate of increase in titratable acidity (%) during growth of *Lactococcus lactis* subsp *cremoris* cultivated in sterilized skim milk supplemented with 1 % peptone and control of acidity at 34°C.

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Table 5. Rate of increase of colony count of *Lactobacillus casei* cultivated at 34°C in sterilized skim milk supplemented with 1 % peptone and control of culture acidity (log CFU/ml).

Sampling time (hour)	Control		Supplemented with 1% peptone and control of culture acidity	
	CFU/ml	Log CFU/ml	CFU/ ml	Log CFU/ml
0	16x 10^5	6.20	178 x 10^4	6.25
2	18 x 10^5	6.25	199 x 10^4	6.29
4	252 x 10^4	6.40	40 x 10^5	6.60
6	40 x 10^5	6.60	90 x 10^5	6.95
8	55 x 10^5	6.74	18 x 10^6	7.25
10	76 x 10^5	6.88	57 x 10^6	7.75
12	11 x 10^6	7.04	90 x 10^6	7.95
24	23 x 10^6	7.36*	17 x 10^7	8.23
26	24 x 10^6	7.38	27 x 10^7	8.43
28	26 x 10^6	7.41	70 x 10^7	8.84

*Coagulated

Table 6. Rate of increase in titratable acidity (%) of *Lactobacillus casei* cultivated at 34°C in sterilized skim milk supplemented with 1% peptone and control of culture acidity.

Sampling time (hour)	Control	Difference in acidity	Supplemented with 1% peptone and control of culture acidity	Difference in acidity
0	0.20	0.00	0.20	0.00
2	0.22	0.02	0.22	0.02
4	0.23	0.03	0.20	0.00
6	0.24	0.04	0.21	0.01
8	0.25	0.05	0.20	0.00
10	0.26	0.06	0.21	0.01
12	0.27	0.07	0.20	0.00
24	0.48*	0.28	0.55	0.35
26	0.50	0.30	0.20	0.00
28	0.51	0.31	0.20	0.00

*Coagulated.

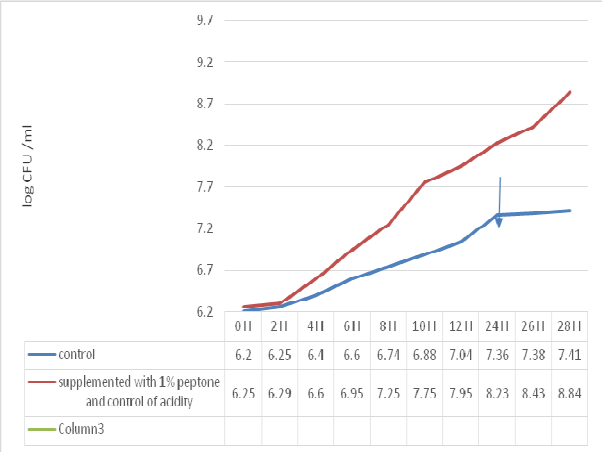

Fig. 5. Rate of increase of colony count of *Lactobacillus casei* cultivatedat 34°C in sterilized skim milk supplemented with 1% peptone and control of culture acidity (log CFU/ml).

↓Coagulated.

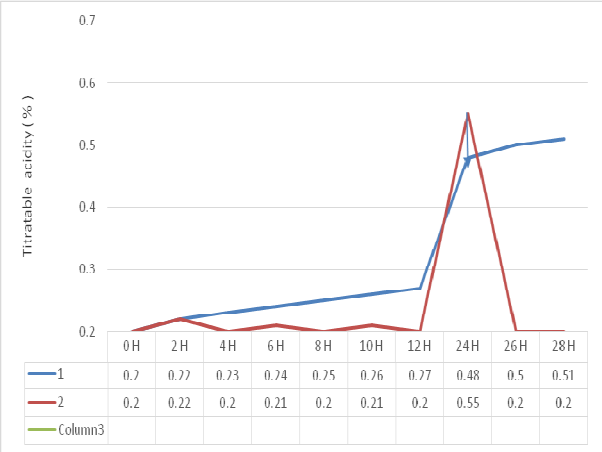

Fig. 6. Rate of increase of titratable acidity (%) of *Lactobacillus casei* cultivated at 34°C in sterilized skim milk supplemented with 1 % peptone and control of culture acidity.

(1) rate of increase of titratable acidity (%) during growth of *Lactobacillus casei* cultivated in sterilized skim milk at 34°C (control).

(2) rate of increase of titratable acidity (%) during growth of *Lactobacillus casei* cultivated in sterilized skim milk supplemented with 1 % peptone and control of acidity at 34°C.

↓Coagulated.

CONCLUSION

Fortification of milk with 1% peptone of controlled acidity yielded an excellent growth for *Lactococcus lactis* sub sp*lactis, Lactococcus lactis* subsp *cremoris* and *Lactobacillus casei,* as the maximum count of colony forming units was obtained.

Thus, sufficient cell mass production in a supplemented medium is possible, and this may allow exclusive use in the dairy industry.

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التأثير المشترك لتدعيم البيئات الغذائيه بالببتون والتحكم فى الحموضه على نمو بكتريا حامض اللاكتيك

داليا جمال ، دينا عثمان و نانيس جمعه وعلى حسن
قسم الألبان – كليه الزراعه- جامعه اسيوط

اجريت هذه الدراسه بغرض التعرف على ومقارنة التاثير المشترك لتدعيم البيئة باستخدام ١% ببتون مع التحكم في درجة حموضة المزرعة على سرعة النمو وسرعة انتاج الحامض لبعض انواع بكتريا حامض اللاكتيك المستخدمة في الصناعات اللبنية عند النمو في لبن فرز معقم على درجة حرارة ٣٤ م.وفي هذه الدراسة استخدمت الانواع البكتيرية الاتية: *Lactococcus lactis* subsp *lactis* - *Lactococcus lactis* subsp *cremoris* - *Lactobacillus casei* وكانت متابعة سرعة النمو عن طريق تقدير عدد الوحدات المكونة للمستعمرات Colony forming units باستخدام بيئة MRS وسرعة تكوين الحامض Developed titratable acidity(DTA).وقد اظهرت النتائج المتحصل عليها:عند تنمية *Lactococcus lactis* subsp *lactis* في بيئة لبن فرز معقم (كنترول) تجبن اللبن بعد ١٢ ساعة من التحضين وكان العدد الاقصى للوحدات المكونة للمستعمرات ٢٨×١٠^{٦} وحدة/مل.ومن ناحية أخري كانت سرعة النمو مقدرة بعدد الوحدات المكونة للمستعمرات او الحموضة المتكونة عند تدعيم البيئة بـ ١% ببتون مع التحكم في حموضة المزرعة واستمرت المزرعة سائلة لم يحدث لها تجبن حتي بعد مرور ٢٨ ساعة على درجة حرارة ٣٤°م. وبلغ العدد الاقصى للوحدات المكونة للمستعمرات ٧٠×١٠^{٦} وحدة/مل بعد ١٢ ساعة من التحضين.وبعد مرور ٢٤ ساعة من التحضن انخفضت سرعة النمو وسرعة تكوين الحامض وبدأ منحنى النمو الدخول في طور الثبات Stationery phase النتائج المقابلة عند استخدام *Lactococcus lactis* subsp *cremoris* نموها في بيئة لبن فرز معقم (كنترول) ووصل عدد الوحدات المكونة للمستعمرات إلي ٥٥×١٠^{٦} وحدة/مل بعد ١٢ ساعة من التحضين.وكانت النتائج المقابلة عند نموها في بيئة لبن فرز معقم مدعم ب ١% ببتون مع التحكم في حموضة المزرعة وبلغ العدد الاقصى للوحدات المكونة للمستعمرات ٢٠×١٠^{٧} وحدة/مل بعد٤٢ ساعة من التحضين.عند نمو بكتريا *Lactobacillus casei* في لبن فرز معقم (كنترول) كان عدد المستعمرات ٢٣×١٠^{٦} مستعمرة/مل. وعند نمو نفس السلالة في بيئة مدعمة بـ ١% ببتون مع التحكم في حموضة المزرعة وبلغ الحد الاقصى للوحدات المكونة للمستعمرات١٧×١٠^{٧} وحدة/مل بعد ٢٤ ساعة من التحضين .ويمكن تلخيص النتائج المتحصل عليها باستخدام ١% ببتون والتحكم في درجة حموضة المزرعة قد ادى الى حدوث زيادة كبيرة في عدد الوحدات المكونة للمستعمرات بالنسبة للانواع البكتيرية الثلاثة المستخدمة ويمكن استخدام هذه النتائج في مصانع الالبان للتحكم في عدد الوحدات المكونة للمستعمرات في البادئات المستخدمة في تصنيع المنتجات اللبنية المختلفة