BEHAVIOUR OF *LACTOBACILLUS ACIDOPHILUS* IN RECONSTITUTED SKIM MILK WITH DIFFERENT SOLID CONTENTS

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Abstract

Acidophilus milk was amde from reconstituted skim milk contaning different concentrations of solids not fat (10, 20 and 30%), heat treated at 90-95°C/30 min, cooled to 37°C, inoculated with 3% *Lactobacilus acidophilus* culture and incubated at 37°C until coagulation. Samples were drawn when frsh and every 2h to determine titratable acidity, pH value and lactose content and examined bacteriologically for total viable, *L. acidophilus* and sporeformers counts. Results revealed that raising the milk solids not fat up to 20% was found to have an increasingly stimulating effect on the behaviour of *L. acidophilus* during fermentation, while, the use of higher levels had lesser effect.

INTRODUCTION

Acidophilus milk is produced by fermentation of milk with *Lactobacillus* acidophilus. This organism is a slow fermenting organism and to get a reasonable fast growth rate, it is customary to heat milk 95°C for 30 minutes or 145°C for 2-3 seconds before inoculation. For viability of the cells, it is preferable to terminate the incubation period when the acidity reaches 0.65%. Continued incubation may result in 1% or more acidity. Acidophilus milk should be cooled to 5°C immediately. Fresh acidophilus milk contains in excess of 500 million cells/ml, although the viable count decreased rapidly with storage (Prescott and Dunnis, 1981).

Recently, many researchers have focused on *L. acidophilus*, as a lactic-acid producing bacteria which is able to implant in the intestines for fermentation to improve the nutritional quality of dairy foods. Consuming acidophilus milk containing large numbers of viable cells may provide benefits in the control of gastrointestinal disturbances (Speck 1975), in restoring the normal intestinal flora which may have been destroyed by antibiotic adminstration (Ramanath *et al.* 1980), in improving lactose digestion which aids lactose in tolerant individuals (Kim and Gilliland 1983), in decreasing sereum cholestrol levels (Grunewald 1985), in increasing vitamin B complex content of the food (Reddy *et al.*, 1976) and more efficient utilization of nutrients (Grunewald and Mitchell 1983).

This work was planned to throw some lights on the behaviour of *L. acidophilus* in reconstituted skim milk of different levels of SNF.

MATERIALS AND METHODS

Imported spray dried skim milk (New Zealand Dairy Board) and fresh skim cows milk were used as shown in the following treatments :

Treatment 1 : Fresh skim cows milk (control).
Treatment 2 : Reconstituted skim milk of 10% SNF.
Treatment 3 : Reconstituted skim milk of 20% SNF.
Treatment 4 : Reconstituted skim milk of 30% SNF.

Milk of all treatments was heat-treated at 90-95^{o}C for 30 minutes, cooled to 37^{o}C and inoculated with a starter culture of *Lactobacillus acidophilus* (Obtained from Chr-Hansens laboratories, Copenhagen Denmark) at the rate of 3%. The inoculated milk was aseptically dispensed in sterile plastic cups and inubated at 37$\pm$1^{o}C until coagulation.

Samples were drown for chemical and bacteriological analysis, when fresh and every 2 hours for 12h. Titratable acidity and pH values were determined according to Ling (1963), pH value was measured using a digital pH meter, and lactose content was estimated using Barnett and Abdel Tawab (1957) method. Total viable bacteria and aerobic sporeformers counts were estimated using the appropiate media as recommended by the American Public Health Association (1978). *L. acidophi-*

Table 1. Some chemical properties of acidophilus milk made from reconstituted skim milk containing different levels of SNF, (Average of 3 replicates).

Incubation time (hour)	Chemical properties of fresh skim cow's milk (control)				Chemical properties of reconstituted skim milk											
					10 % SNF				20 % SNF				30 % SNF			
	Acidity %	pH value	Lactose %	Lactose hydro-lysis%	Acidity %	pH value	Lactose %	Lactose hydro-lysis%	Acidity %	pH value	Lactose %	Lactose hydro-lysis%	Acidity %	pH value	Lactose %	Lactose hydro-lysis%
0	0.19	6.35	4.70	--	0.18	6.40	4.85	--	0.35	6.30	4.45	--	0.57	6.25	14.25	--
2	0.25	6.20	4.65	1.06	0.21	6.16	4.82	0.62	0.41	6.10	9.36	0.95	0.61	6.20	14.15	0.70
4	0.32	5.90	4.58	2.55	0.33	5.97	4.74	2.27	0.48	5.96	9.20	2.65	0.67	6.09	13.97	1.96
6	0.51	5.56	4.37	7.02	0.54	5.58	4.60	5.16	0.72	5.62	9.02	4.55	0.83	5.79	13.75	3.51
8	0.56	5.35	4.26	9.36	0.60	5.30	4.41	9.07	0.90	5.36	8.78	7.08	0.96	5.64	13.43	5.75
10	0.73*	5.0	4.03	14.26	0.75*	5.06	4.19	13.61	1.15*	5.08	8.48	10.26	1.08	5.45	13.18	7.51
12	0.82	4.92	3.95	15.96	0.86	4.90	4.11	15.26	1.25	4.95	8.32	11.96	1.15*	5.25	12.87	9.68

lus was enumerated according to Hull and Roberts (1984) using modified MRS agar.

RESULTS AND DISCUSSION

1. Chemical analysis

1.1 Acid production :

Results in Tables 1 & 2 indicate that the titratable acidity (TA) varied, at zero time, from one treatment to another owing to variation in the chemical composition of the milk. During the first 4h, TA and rate of acid production (R.A.P) increased slowly in all treatments, then, it increased rapidly up till milk coagulation (10h for treatments 1, 2 and 3 and 12h for treatment 4). Prasad and Gandhi (1987) noticed also that, the increase in the acidity in *L. acidophilus* R culture was observed after 4h of incubation, and the percent of lactic acid was constant even after 96h of incubation.

Table 2. Rate of acid production of acidophilus milk made from reconstituted skim milk of different levels of SNF.

Incubation Period (h)	Fresh skim cows milk (control)	Rate of Acid production in skim milk		
		10% SNF	20% SNF	30% SNF
2	0.06	0.03	0.06	0.04
4	0.13	0.15	0.13	0.10
6	0.32	0.36	0.37	0.26
8	0.37	0.42	0.55	0.39
10	0.54	0.57	0.80	0.51
12	0.63	0.68	0.90	0.58

Moreover, T.A and RAP, for all treatments, increased during the first 8h of

incubation as the SNF content of the milk increased (Tables 1&2). The greatest increase in TA and RAP was recorded in treatment 3 (20% SNF) at the end of the incubation period. Final acidity attained at the end of the incubation period in treatment 1 and 2 (each of 10% SNF content) ranged between 0.82- 0.86%, and was found nearly similar to that obtained by Aziz (1992).

In general, it was observed that increasing milk SNF up to 20% appeared to have an increasingly stimulating effect on the TA and RAP of the resulting acidophilus milk. This is slightly contradictory with that found by Pyatkova and Lukovunikova (1974) who stated that milk containing 18-30% TS had an increasingly stimulating effect on *L. acidophils* growth, while, 36 and 42% had an inhibitory effect. The hampered acidity developed at higher SNF content may be attributed to the increased buffering capacity as well as to the retarded growth of the culture organism on account of osmotic pressure.

1.2. pH Value :

pH values in the treatments behaved in an opposite trend to the TA along the incubation period (Table 1). At zero time, these values were affected by the SNF content of the milk, so, a reverse relationship was found between them. With the advancement in the incubation period, these values were decreased gradually in all treatments till the end of incubation. This decrease was found to be higher in treatments 1, 2 and 3 than in treatment 4. Variations in milk SNF and their effect on the viability and growth of *L. acidophilus* were probably responsible for that. Results also, show that treatments 1, 2 and3 have nearly similar values of pH (4.9-4.95) at the end of the incubation period compared with treatment 4.

1.3. Lactose content :

Lactose hydrolysis by L. acidophilus in relation to SNF content of the milk was presented in Table 1. It was observed that, the rate of lactose hydrolysis increased in all treatments, as the incubation period proceeded and the higher rate of hydrolysis was found in treatments 1 (cont) and 2. Up to 4h, this hydrolysis started in slow rate in the four treatments, then, increased in variable levels. The maximum rate of hydrolysis recorded after 12h of incubation ranged between 15-16% for treatments 1 and 2, whereas treatments 3 and 4 recorded 9-11%. These findings conicide with those reported by Noeman and Shalaby (1992) who stated that the greatest decrease

in lactose content of acidophilus milk during fermentation, was from about 4.73 g/100g in base milk (unfermented milk) to about 4.07 g/100g in acidophilus milk (13.95% lactose hydrolysis).

It is valuable to mention that, although the rate of hydrolysis decreased as the SNF content of the milk increased, the amount of utilized lactose in the four treatments behaved in another trend and was found to be directly proportional with the SNF content of the milk.

2. Bacteriological examination

2.1. Total viable and *L. acidophilus* counts :

Counts of total viable bacteria (TC) and *L. acidophilus* increased gradually in all treatments as the incubation period advanced (Table 3). During the first 4h, *L. acidophilus* counts were higher in treatment 1 (cont.) than in the other treatments. As the incubation period extended, these counts declined much in control treatment than in the corresponding ones of reconstituted skim milk. A relationship between milk SNF content and counts of *L. acidophilus* and TC during the fermentation period, may be noticed. So, the use of ascending levels of SNF (up to 20%) seemed to have a stimulating effect on the growth and counts of these roganisms, whereas, the use of higher levels exhibited a slight inhibitory effect. The highest number of *L. acidophilus* was recorded in treatments 3 (88×10^7 cFu/g), after 12h of incubation, while, the lowest one (58.5×10^7 cFu/g) belonged to control treatment. These results were higher than those mentioned by Rao and Gandhi (1988) who stated that the highest numbers of total viable *L. acidophilus* (16×10^7 cFu.ml), and the greatest antimicrobial properties were obtained with homogenized sterilized skim milk acidophilus milk than that made from cow or buffaloes milk (5 or 10% fat).

Data in addition, pointed out that counts of TC and *L. acidophilus* of control treatment were approximately near to those of treatment 2, at later stages of incubation, and the same observation was found also between the remaining 2 treatments 3 and 4. These counts of *L. acidophilus* ranged between 10^6-10^7 cFu/g. Furthermore, it was noticed that, the trend of change in *L. acidophilus* counts during the fermentation period was similar to that of TC in all treatments. These organisms were gained mainly to acidophilus milk by 2 main sources, skim milk powder which quoted between thousands and millions per gramme (Robinson, 1981) and *L. acidoph-*

ilus culture.

2.2. Sporeformers counts :

At the beginning of the incubation period, it was observed that the counts of sporeformers were relatively lower in treatments 1 and 2 than in the others owing to the low SNF content presented in these treatments (Table 3). As the incubation period progressed, these counts increased in all treatments up to 4h then, decreased gradually till the end of incubation period. This increase of sporeformers counts was presumably due to some reasons as decreasing of the acidity of all treatments at the begining (at zero time), *L. acidophilus* was not the predominant organism at this period,using heat-treatment for milk instead of sterilization, and presence of the spores of these organisms initially in the skim milk powder (Keogh, 1971).

Decrease in sporeformers counts, after 4h of incubation, could be attributed to the production of antimicrobiol substances (antibiotics, H_2O_2 and lactic acid) which accumulated during the initial stages of acidophilus milk fermentation (Gilliland and Speck 1972 and Sukhova *et al.* 1978). On the other hand, Prasad and Gandhi (1987) stated that the antimicrobial activity of *L. acidophilus* R strain found only after 12h of incubation at 37°C reached the maximum activity at pH 3.2 and decreased with an increase in pH. They added that no inhibition zone was observed at pH above 4.5. This means that, the antimicrobial effect is greatly related to the low pH.

Generally, it could be concluded that, the bebehaviour of *L. acidophilus* in reconstituted skim milk of different concentrations of SNF was apparently enhanced in the milk containing up to 20% SNF, whereas, the use of higher levels had a slight effect. This is in agreement with the higher TA, RAP and the greatest counts of viable *L. acidophilus* noticed in that milk (20% SNF).

Table 3. Total viable (TC), L. acidophilus (L.A.) and sporeformers (SP) counts of acidophilus milk made from reconstituted skim milk of different levels of SNF, (Average of 3 replicates).

Incubation period (hr)	Bacteriological properties of fresh skim cow's milk (control)			Bacteriological properties of reconstituted skim milk								
				10 % SNF			20 % SNF			30 % SNF		
	TC $x10^7$	LA $x10^7$	SP x10	TC $x10^7$	LA $x10^7$	SP x10	TC $x10^7$	LA $x10^7$	SP x10	TC $x10^7$	LA $x10^7$	SP x10
0	5.1	7.4	2.2	4.7	6.2	2.5	4.9	6.0	4.0	4.3	6.5	5.0
2	6.0	8.1	3.5	5.4	7.5	3.2	5.5	7.6	6.5	4.6	7.2	8.0
4	7.0	10.3	4.5	7.8	8.1	4.0	6.6	8.4	8.6	6.6	8.5	10.0
6	13	17.3	3.3	14.2	19.2	3.5	15.8	18.6	5.5	14.5	16.2	8.5
8	18.6	28.5	2.3	21.4	35	2.5	25.5	41.5	4.0	22.2	34	6.2
10	32	42.5	2.0	39	50.5	1.3	53	81	3.2	46	62	5.3
12	43	58.5	1.4	51	63	1.0	60.5	88	2.5	56	71	4.0

REFERENCES

1. American Public Health Association, A.P.H.A. 1978. Standard methods for examination of dairy products. American Public Health Association. New York.
2. Aziz, A. H. H. 1992. Studies on some fermented milk. Thesis, M. Sc. Fac. Agric. Cairo Univ., Egypt.
3. Barnett, A. G. and Abd El-Tawab. 1957. A rapid method for the determination of lactose in milk and cheese. J. Sci. Food Agric., 7 : 437-441.
4. Gilliland, S. E. and M. L. Speck. 1972. J. Milk food Technology. 35 : 307.
5. Grunewald, K. K. 1985. Influence of bacterial starter cultures on nutritional value of food : Effect of *L. acidophilus* fermented milk on growth and serum cholesterol in laboratory animals. Cultured dairy products. J., 20 (4) : 24.
6. Grunewals and Mitchells. 1985. Growth of mice fed milk fermented with *L. acidophilus*. J. Food prot., 46 : 315.
7. Hull, R. R. and A. V. Roberts. 1984. Differential enumeration of *L. acidophilus* in yoghurt. Aust. J. Dairy Tech., 39 : 164.
8. Keogh, B. P. 1971. J. Dairy Res., 38 (1) : 91-111.
9. Kim, H. S. and S. E. Gilliland. 1983. *Lactobacillus acidophilus* as a dietary adjunck for milk to aid lactose digestion in humans. J. Dairy Sci., 66 : 959.
10. Ling, E. R. 1963. A text book of Dairy chemistry. Vol. 2, 3rd Ed. Chapman and Hall Ltd. London.
11. Noeman, A. A. and S. O. Shalaby. 1992. A comparative study between zabady and acidophilus milk I. Nutritional value. Egypt. J. Food Sci., 20, Suppl., P. 43-51.
12. Prasad, r. V. and D. N. Gandhi. 1987. Factors affecting the production of antibacterial substances in *Lactobacillus acidophilus* strain R. Ind. J. Dairy Sci., 40, 1, 1987.
13. Prescott and Dunnis. 1981. Industerial Microbiology. 4th Ed. Avi. Publishing Company, Wisconsin, U.S.A.
14. Pyatkova, N. P. and L. A. Lukovunikova. 1974. Development of *Lactobacillus acidophilus* in skim milk. Molochanya promyshlennost, 79 : 63. (c.f. DSA 42 : 2124).
15. Ramanath, K. R., H. Laxminarayana and K. Dayaln. 1980. Effect of oral adminstration of tetracycline and cultures of *L. acidophilus* on intestinal microflora of rats. Mysore J. Agric. Sci., 14 : 373. c.f. Cultured Dairy Products

J., 20 (4) : 17.

16. Rao, S. M. and D. N. Gandhi. 1988. Studies on various quality characteristics of acidophilus sour milk from buffalo milk. Cultured Dairy products. J., 23 (2) : 21.
17. Reddy, K. P., K. M. Shahani and S. M. Kulkarni. 1976. B-complex vitamins in cultured and acidified yoghurt. J. Dairy Sci., 59 : 191.
18. Robinson, P. K. 1981. Dairy Microbiology. Vol. I. The microbiology of milk. applied Sci. Publishers. London and New Jersey.
19. Speck, M. L. 1975. Market outlook for acidophilus food products. Cultured Dairy products. J., 10 : 8.
20. Sukhova, T. S., L. V. Ivanova, A. N. Bulalskuya, T. V. Knyazeva and Z. A. Biryukova. 1978. Study of the antibiotic activity of acidophilus products for infant feeding. In xx International Dairy Congress Vol, E. 970-971. (c.f. DSA (1978) Vol. 40, 4918.

دراسة سلوك *Lactobacillus acidophilus* فى اللبن الفرز المسترجع المحتوى على نسب مختلفة من المواد الصلبة اللادهنية

عادل على قنديل ، شفيقة عبد القادر ، سعاد شريف

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تم معاملة اللبن الفرز المسترجع المحتوى على ١٠ ، ٢٠ ، ٣٠٪ مواد صلبة لا دهنية واللبن البقرى الفرز الطازج ، بالحرارة على ٩٠-٩٥ °م لمدة نصف ساعة ... ثم التبريد الى ٣٧ °م . أضيف البادىء *L. acidophilus* الى كل منهما بنسبة ٣٪ ثم التحضين على ٣٧ °م حتى التجبن ... وقد أظهرت النتائج ما يلى :

– إختلفت نسبة الحموضة ورقم الـ pH فى بداية التحضين بإختلاف نسبة المواد الصلبة اللادهنية .

– كان لزيادة نسبة المواد الصلبة اللادهنية فى اللبن حتى ٢٠٪ تأثير منشط على نسبة الحموضة وعلى معدل إنتاج الحموضة خلال مدة التحضين ، فى حين كان تأثير نسبة الـ ٣٠٪ أقل .

– تراوحت نسبة الحموضة فى اللبن المحتوى على ١٠٪ مواد صلبة لا دهنية بين ٠,٨٢ – ٠,٨٦ ٪ فى حين كانت هذه النسبة ١,٢٥ ٪ فى اللبن المحتوى على ٢٠٪ مواد صلبة لادهنية .. فى نهاية مدة التحضين .

– إنخفضت النسبة المئوية للاكتوز المتحلل بزيادة نسبة المواد الصلبة اللادهنية فى حين كانت كمية اللاكتوز المتحللة عكس ذلك.

– لم يتم تحليل سوى ١٥ – ١٦ ٪ من محتوى اللاكتوز فى اللبن المحتوى على ١٠٪ مواد صلبة لا دهنية فى حين كانت هذه النسبة ٩ – ١١٪ فى اللبن المحتوى على ٢٠ ، ٣٠٪ مواد صلبة لا دهنية وذلك فى نهاية مدة التحضين .

– كان لزيادة نسبة المواد الصلبة اللادهنية حتى ٢٠٪ تأثير منشط على نمو *L. acidophilus* وكان هذا التأثير بدرجة أقل مع زيادة المواد الصلبة اللادهنية .

– تراوحت الأعداد الكلية للبكتيريا وأعداد *L. acidophilus* ما بين ١٠ ^{٦} ، ١٠ ^{٧} خلية / جم فى جميع المعاملات طوال مدة التحضين .

– كان أكبر عدد لبكتيريا *L. acidophilus* بعد ١٢ ساعة من التحضين ٨٨ X ١٠ ^{٧} خلية / جم فى اللبن المحتوى على ٢٠٪ مواد صلبة لا دهنية فى حين كان أقل عدد ٥٨,٥ X ١٠ ^{٧} خلية / جم فى لبن المقارنة .

– إختلف عدد البكتيريا المكونة للجراثيم فى بداية التحضين بإختلاف نسبة المواد الصلبة اللادهنية ثم إزداد عددها فى جميع المعاملات حتى ٤ ساعات من التحضين ثم إنخفض تدريجياً بعد ذلك بسبب زيادة النسبة المئوية للحموضة والمواد المضادة لنموها .