THE EFFECT OF MILK SUPPLEMENTATION ON THE DEVELOPMENT AND ACIDIFICATION OF LACTIC BACTERIA

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ABSTRACT

Influence of yeast extract supplementation on the development and acidification of 4 strains of lactic bacteria; *Streptococcus lactis, Streptococcus cremoris, Lactobacillus casei* and *Lactobacillus acidophilus* were investigated in pasteurised skimmed milk. Three types of yeast extract, M-normal yeast extract, N-yeast supplemented with several vitamins, and O-yeast extract supplemented with group B vitamins, were employed at concentrations of 0.05%, 0.2%, 0.5% and 1.0%. Milk without yeast extract was used as control. Test cultures were inoculated into the various milk products and incubated at 37^0C. Total cell counts (cfu/ml) and total titratable acidity (% lactic acid) was estimated at 0,3 and 6 hours intervals. The results showed that both cell counts and acidity increased proportionally with increased levels of yeast extract supplementation. Yeast extract could be used to remedy fermentation problems encountered in milk industry.

Key words: Lactic bacteria, yeast extract, supplementation, milk, and development.

INTRODUCTION:

Lactic bacteria are microorganisms, which in mixed or pure cultures are capable of multiplying in the bossom of milk and curds, to produce at favourable moments, the acidity and aroma sought in the milk industry (Kothari and Nambudripad, 1973). These microorganisms take part largely in the process of the preparation and the maturation of milk products like yoghurts, cheese, butter, etc., and their role lies in the glycolysis,

proteolysis, lipolysis, production of aroma constituents (diacetyl and acetoin) and the effect of inhibiting or stimulating of other microorganisms (Bautista, *et al.*, 1966; Brinkten, *et al.*, 1994; Graciela, *et al.*, 1995). They are the natural contaminants of milk and they produce almost uniquely lactic acid from sugar lactose. Acidity of fermented milk is sufficient to prevent spoilage by proteolytic or other bacteria that are not acid-tolerant (Frazier and Westhoff, 1978). In the milk industry, the lactic bacteria are chosen on the basis of their activity, that is their ability to produce more or less, large quantities of lactic acid in a given time interval and the fact that they are non-pathogens. Their ability to produce lactic acid varies depending on the strain and conditions for growth. The two principal bacterial species, which intervene in the process of lactic fermentation, belong to the group *Streptococcus* and the *Lactobacillus* and are further divided into subgroups. These lactic bacteria are often exigent for their nitrogenous and vitamin nutrients. They are auxotrophic for certain vitamins, amino acids, peptides, and at times purines and pyrimidines (Bracquart and Lorient, 1977; Olmos-Dichara, *et al.*, 1997; Amrane and Prigent, 1998), which limit their multiplication to a small number of favourable media. The presence of these free growth factors in the medium will enhance the multiplication of these bacteria. One of the natural medium in which the lactic bacteria grow is milk. Milk contains a complete range of nutritive elements (glucose, lactose, natural amino acids, minerals and vitamins) that are essential to support growth of lactic bacteria.

The milk industries are often faced with the problem of variation of milk composition. This may arise as a result of adulteration of the milk by practices external to the milk either by accident or design, by the addition of extraneous water and chemical substances (detergents, preservatives, disinfectants, feeds etc) as well as adulterations entering the milk through the cow itself (colostrums, blood, drugs etc) (Early, 1998). Such chemical substances often have bactericidal/bacteriostatic effect on starter cultures. Also, crude milk contains certain quantities of bacteria (with stimulating effects) and several growth factors naturally pre-existing in it. During processing, sterilization or pasteurisation temperatures remove or destroy some of these thermolabile growth factors, thereby depleting the milk of nutritive substances. Milk with high levels of adulterants or low amount of growth factors would sluggishly support the performances of starter cultures (Early, 1998). This constitutes a defect called "Lazy" milk in dairy industry. There is

therefore the need to supplement such milk with more micronutrients to enhance the performances of starter cultures.

Yeast extract is a substance that is complex with high nutritive value. Besides the vitamin B group, it is rich in peptides and amino acids (Smith *et al* 1975). Such a substance could be used to augment nutrients in milk.

It is therefore the objective of this study to determine the effect of supplementation of milk with yeast extracts on the growth and acidification of lactic bacteria.

MATERIALS AND METHODS

Materials

The Industrial strains of *Streptococcus lactis; Streptococcus cremoris, Lactobacillus casei*: and *Lactobacillus acidophilus* were provided by Societe Callixte-France; Three yeast extracts N-normal yeast extract, M-yeast extract supplemented with several vitamins and O – yeast extract supplemented with group B vitamins, were provided by Lacto Labo-France. Growth medium was Elliker medium (DIFCO 097401) for cell count and acidity determination. Culture medium for the four strains of lactic bacteria was Eugon broth medium (Biomerieux 51041). Pasteurised skimmed milk was employed in the investigation while milk without yeast extract was used as control.

Microbiological Analysis

The tests were carried out by aseptically inoculating the pasteurised skimmed milk containing the three different yeast extracts at four different concentrations (0.05%, 0.2%, 0.5% and 1%) with 20% test culture organisms (2 ml of culture in Eugon broth) in 10 ml of milk. The test samples were incubated at 37^0C; cell counts and total titratable acidity were estimated at 0, 3 and 6 hours intervals.

Cell Count

Total viable aerobic plate count (APC) was carried out using pour plate method as described by Harrigan and McCance (1979).

Triplicate 1 ml test samples were aseptically transferred (at appropriate intervals) into 9 ml sterile distilled water as dilute in sterilized test tubes. This was followed by appropriate serial dilutions of the test samples. 1 ml of each dilution was aseptically transferred into sterile petri dishes and was over laid with molten Elliker medium, which had been cooled down to 45^0C. The plates were gently swirled and allowed to solidify. The plates were incubated in inverted positions at 37^0C for a period of 24 hours after which cell counts were determined by multiplying the number of colonies counted by the dilution factors. The results were expressed as colony forming units (cfu/ml) per ml of sample.

Total titratable acidity (TTA)

Total titratable acidity was determined by method described by AOAC (1990).

Triplicate 10ml test samples were titrated against 0.1N NaOH using phenolphthalein as indicator. Percentage total titratable acidity was calculated as % lactic acid.

Statistical Analysis

Analysis of variance (ANOVA) was performed on the total cell count (cfu/mL) and total titratable acidity (TTA) using the methods of Steel and Torrie (1980). The least significant differences (LSD) were calculated using Tukey's test multiple comparisons (Steel and Torrie, 1980).

RESULTS AND DISCUSSIONS

Table 1 shows influence of various yeast extracts on the growth of *S. lact*

Table 1: Influence of Various Yeast Extracts on growth of *S. lactis* in skimmed milk

Concentration of yeast extract. (%)														
		0.05			0.2			0.5			1.0			
Milk Samples	Time (hrs) cfu/ml	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1	t_2	
MM		$1.00x10^{4a}$±0.54	$2.5x10^{8b}$±0.06	$4.0x10^{8c}$±0.84	$1.0x10^{4A}$±0.12	$3.0x10^{8C}$±0.81	$4.5x10^{8C}$±0.41	$1.0x10^{4a}$±0.07	$3.5x10^{4b}$±0.61	$4.5x10^{8b}$±0.51	$1.0x10^{4b}$±0.04	$4.0x10^{8b}$±0.07	$5.0x10^{8c}$±0.12	
MN		$1.20x10^{4a}$±0.03	$3.5x10^{8a}$±0.02	$4.5x10^{8a}$±0.12	$1.2x4^{a}$±0.01	$4.0x10^{8b}$±0.09	$5.0x10^{8c}$±0.22	$1.2x10^{4a}$±0.00	$5.0x10^{8a}$±0.08	$5.8x10^{8c}$±0.01	$1.2x10^{8a}$±0.11	$6.0x10^{8a}$±0.01	$1.5x10^{9nc}$±0.10	
MO		$1.10x10^{4a}$±0.01	$3.5x10^{8a}$±0.00	$4.5x10^{8a}$±0.03	$1.1x10^{4a}$±0.03	$4.5x10^{8a}$±0.31	$6.0x10^{8a}$±0.01	$1.1x10^{4a}$±0.01	$5.0x10^{8a}$±0.11	$5.7x10^{8a}$±0.03	$1.1x10^{4b}$±0.00	$6.0x10^{8a}$±0.23	$8.0x10^{8b}$±0.21	
M		$1.0x10^{4a}$±0.00	$6.0x10^{4c}$±0.17	$2.0x10^{5b}$±0.01										
LSD		$2.3x10^{4}$	$1.2x10^{4}$	$3.9x10^{7}$	$1x10^{2}$	$4.2x10^{7}$	$5.0x10^{7}$	$2.1x10^{2}$	$4.7x10^{8}$	$2.3x10^{8}$	$1.17x10^{8}$	$1.85x10^{8}$		

Means not followed by the same superscript in the same column are significantly different from each other at 5% probability level.

± = Standard deviation of triplicate analysis

LSD = Least Significant difference

MM= Milk +Extract M

MN = Milk +Extract N

MO = Milk +Extract O

M = Milkon

The growth increased with increase in yeast extract concentrations. N–yeast extract showed greater growth stimulation, followed by 0-yeast extract.

Table 2. Shows influence of various yeast extracts on the growth of *S.cremoris*.

Table 2: Influence of Various Yeast Extracts on growth of *S.cremoris* in skimmed milk

Concentration of yeast extract. (%)													
		0.05			0.2			0.5			1.0		
Milk Samples	Time (hrs) cfu/ml	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1	t_2
MM		$2x10^{4a}$±0.0	$5.5x10^{6b}$±0.71	$2.0x10^{7c}$±0.01	$1.2x10^{4a}$±0.08	$7.0x10^{7b}$±0.03	$2.0x10^{8C}$±0.01	$1.2x10^{4a}$±0.00	$1.0x10^{8b}$±0.03	$2.5x10^{8c}$±0.01	$1.2x10^{4a}$±0.01	$1.5x10^{8c}$±0.10	$3.0x10^{8}$±0.01
MN		$1.1x10^{4a}$±0.1	$1.5x10^{8a}$±0.03	$2.5x10^{8b}$±0.05	$1.1x10^{4a}$±0.01	$2.0x10^{8a}$±0.40	$3.0x10^{8a}$±0.04	$1.1x10^{4a}$±0.04	$2.5x10^{8a}$±0.10	$5.5x10^{8a}$±0.05	$1.1x10^{4a}$±0.00	$3.0x10^{8a}$±0.1	$6.0x10^{8b}$±0.70
M0		$3x10^{4a}$±0.0	$1.5x10^{8a}$±0.03	$3.0x10^{8a}$±0.00	$1.3x10^{4a}$±0.01	$1.9x10^{8a}$±0.77	$2.5x10^{8b}$±0.05	$1.3x10^{4a}$±0.11	$2.3x10^{8a}$±0.35	$5.0x10^{8b}$±0.10	$1.3x10^{4a}$±0.04	$2.0x10^{8b}$±0.0	$9.0x10^{8}$±0.13
M		$1.5x10^{4a}$±0.14	$1.5x10^{4c}$±0.02	$1.2x10^{5d}$±0.16									
LSD		$1.05x10^{2}$	$1.32x10^{6}$	$1.17x10^{7}$	$9x10^{2}$	$1.25x10^{8}$	$4.25x10^{8}$	$1.01x10^{2}$	$1.93x10^{7}$	$2.21x10^{8}$	$9.3x10^{2}$	$4.1x10^{7}$	$2.5x10^{8}$

Means not followed by the same superscript in the same column are significantly different from each other at 5% probability level.

± = Standard deviation of triplicate analysis

LSD = Least Significant difference

MM= Milk +Extract M

MN = Milk +Extract N

MO = Milk +Extract O

M = Milk only

Growth of this organism drastically increased with increase in yeast extract levels. O– yeast extract had greater influence on the growth rate of *S. cremoris*, followed by N-yeast extract. M-yeast extract had least effect on the growth of this organism.

Table 3 shows influence of various yeast extracts on the growth of *L. casei*.

Table 3: Influence of Various Yeast Extracts on growth of *L. casie* in skimmed milk

Concentration of yeast extract. (%)														
		0.05			0.2			0.5			1.0			
Milk Samples	Time (hrs) cfu/ml	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1	t_2	
MM		$1.1x10^{4a}$±0.03	$1.0x10^{6a}$±0.18	$2.5x10^{8a}$±0.51	$1.1x10^{4a}$±0.22	$1.5x10^{6a}$±0.10	$3.0x10^{8a}$±0.01	$1.1x10^{4a}$±0.94	$1.6x10^{6a}$±0.05	$3.5x10^{8a}$±0.02	$1.1x10^{4a}$±0.16	$2.0x10^{6a}$±0.70	$5.0x10^{8a}$±0	
MN		$1.0x10^{4a}$±0.01	$2.0x10^{6a}$±0.05	$3.0x10^{8a}$±0.06	$1.0x10^{4a}$±0.09	$2.5x10^{6a}$±0.16	$4.0x10^{8a}$±0.00	$1.0x10^{4a}$±0.07	$2.5x10^{6a}$±0.13	$4.5x10^{8a}$±0.01	$1.0x10^{4a}$±0.02	$3.0x10^{6a}$±0.07	$5.0x10^{8a}$±0	
MO		$1.2x10^{4a}$±0.05	$1.3x10^{6a}$±0.02	$2.5x10^{8a}$±0.00	$1.2x10^{4a}$±0.13	$3.0x10^{6a}$±0.04	$4.0x10^{8a}$±0.01	$1.2x10^{4a}$±0.26	$2.6x10^{6a}$±0.05	$3.0x10^{8a}$±0.18	$1.2x10^{4a}$±0.00	$2.6x10^{6a}$±0.01	$3.5x10^{8a}$±0	
M		$1.5x10^{3a}$±0.00	$2.0x10^{5a}$±0.11	$2.5x10^{7a}$±0.11										
LSD		$8.5x10^{3}$	$6.5x10^{5}$	$3x10^{8}$	$9x10^{3}$	$5.5x10^{5}$	$9.5x10^{7}$	$8.21x10^{3}$	$8.56x10^{5}$	$9.91x10^{7}$	$9.0x10^{3}$	$2.5x10^{6}$	$1.0x10^{8}$	

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± = Standard deviation of triplicate analysis

LSD = Least Significant difference

MM = Milk + Extract M

MN = Milk + Extract N

MO = Milk + Extract O

M = Milk only

0.5% of all the three yeast extracts showed no significant difference in the cell counts at 6 hours incubation. At 1% yeast concentration, M and N – yeast extracts had similar values (5.0 x 10^8 cfu/ml) at 6 hours, while 0-yeast extract had lower cell count – 3.5 x 10^5 cfu/ml. Nevertheless, increased yeast extract levels resulted in increase in cell count of all test samples.

Table 4 shows influence of various yeast extracts on the growth of *L. acidophilus*.

Table 4: Influence of Various Yeast Extracts on growth of ***L.acido*** in skimmed milk

Concentration of yeast extract. (%)													
		0.05			0.2			0.5			1.0		
Milk Samples	Time (hrs) cfu/ml	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1	
MM		$1.0x10^{4e}$±0.92	$1.5x10^{5c}$±0.50	$3.0x10^{6a}$±0.08	$1.0x10^{4a}$±0.00	$2.0x10^{5b}$±0.01	$3.5x10^{5b}$±0.09	$1.0x10^{4b}$±0.02	$2.3x10^{5b}$±0.01	$4.0x10^{5c}$±0.00	$1.0x10^{4a}$±0.03	$2.5x10^{5b}$±0.19	
MN		$1.0x10^{4e}$±1.61	$1.0x10^{5c}$±0.02	$3.0x10^{6a}$±0.11	$1.0x10^{4a}$±0.06	$1.9x10^{5b}$±0.09	$3.8x10^{5b}$±0.06	$1.0x10^{4b}$±0.01	$2.5x10^{5b}$±0.43	$4.8x10^{5c}$±0.82	$1.0x10^{4a}$±0.01	$1.5x10^{5c}$±0.02	
MO		$1.3x10^{4de}$±0.06	$2.0x10^{5c}$±0.10	$3.0x10^{6a}$±0.02	$1.3x10^{4a}$±0.17	$3.0x10^{5a}$±0.74	$4.5x10^{5a}$±0.01	$1.8x10^{4a}$±0.07	$3.0x10^{5a}$±0.14	$5.0x10^{5a}$±3.02	$1.3x10^{4a}$±0.10	$3.7x10^{5a}$±0.75	
M		$1.5x10^{4d}$±0.12	$2.5x10^{4d}$±0.01	$2.7x10^{4b}$±0.14									
LSD		$1.15x10^3$	$4.5x10^3$	$2.5x10^3$	$9.1x10^2$	$8.57x10^4$	$6.5x10^3$	$7.5x10^3$	$4.3x10^3$	$1.9x10^3$	$9.2x10^2$	$9.7x10^3$	

Means not followed by the same superscript in the same column are significantly different from each other at 5% probability level.

± = Standard deviation of triplicate analysis

LSD = Least Significant difference

MM= Milk +Extract M

MN = Milk +Extract N

MO = Milk +Extract O

M = Milk only

The cell counts increased with increase in yeast extract levels for all the different yeast extracts. O – yeast extract had greater effect on *L. acidophilus* followed by M-yeast extract. However at 0.05% yeast extract concentration, there was no significant difference in the cell count among the three yeast extracts at 6 hours incubation, though all values were superior to the value of the control (2.7×10^4 cfu/ml).

Table 5 shows the influence of various yeast extracts on the acidification of *S. lactis*.

Table 5: Influence of Various Yeast Extracts on acidification of *S. lactis* in skimmed milk

Concentration of yeast extract. (%)												
		0.05			0.2			0.5			1.0	
Milk Samples	Time (hrs) % lactic acid	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1
MM		10.00^a±0.06	12.50^b±0.04	21.25^b±0.01	10.10^a±0.00	13.75^a±0.01	22.50^b±0.01	10.21^a±0.01	15.00^c±0.03	24.50^b±0.0	10.25^a±0.03	16.25^a±0.00
MN		10.00^a±0.00	12.50^b±0.09	21.75^b±0.05	10.12^a±0.00	12.25^b±0.04	24.25^a±0.01	10.20^a±0.07	13.00^b±0.12	25.00^a±0.0	10.25^a±0.01	13.75^b±0.02
MO		10.05^a±0.01	14.03^a±0.05	22.50^a±0.30	10.12^a±0.06	11.50^c±0.01	22.50^b±0.16	10.10^a±0.03	15.25^a±0.00	22.50^c±0.0	10.15^a±0.01	16.00^a±0.21
M		10.00^a±0.02	11.50^c±0.00	13.75^c±0.23								
LSD		0.12	0.50	0.32	0.09	0.63	1.13	0.12	1.94	0.33	0.95	1.71

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± = Standard deviation of triplicate analysis

LSD = Least Significant difference

MM= Milk +Extract M

MN = Milk +Extract N

MO = Milk +Extract O

M = Milk only

The total titratable acidity produced by this lactic bacterium increased with increased levels of the different yeast extracts. N – yeast extract showed greater effect, followed by M-yeast extract. However at 1% yeast extract concentration there was no significance differences in the acidity between M and O yeast extracts at 6 hours incubation.

Table 6 shows influence of various yeast extracts on the acidification of *S. cremoris*.

Table 6: Influence of Various Yeast Extracts on acidification of *S. cremoris* in skimmed milk

Concentration of yeast extract. (%)													
		0.05			0.2			0.5			1.0		
Milk Samples	Time (hrs) % lactic acid	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1	t_2
MM		$10.00^a \pm 0.13$	$10.50^b \pm 0.03$	$10.60^c \pm 0.22$	$10.12^a \pm 0.01$	$10.60^a \pm 0.27$	$11.25^a \pm 0.00$	$10.15^a \pm 0.02$	$10.60^a \pm 0.07$	$11.25^b \pm 0.04$	$10.25^a \pm 0.00$	$11.25^a \pm 0.01$	$13.75^b \pm 0.16$
MN		$10.00^a \pm 0.05$	$10.25^b \pm 0.01$	$11.50^b \pm 0.01$	$10.11^a \pm 0.11$	$10.75^a \pm 0.16$	$11.25^a \pm 0.02$	$10.12^a \pm 0.01$	$10.80^b \pm 0.06$	$13.25^a \pm 0.51$	$10.15^a \pm 0.19$	$11.50^a \pm 0.11$	$16.25^a \pm 0.00$
M0		$10.10^a \pm 0.00$	$10.75^b \pm 0.05$	$11.25^b \pm 0.00$	$10.12^a \pm 0.42$	$10.75^a \pm 0.42$	$11.25^a \pm 0.06$	$10.12^a \pm 0.05$	$10.30^a \pm 0.65$	$11.25^b \pm 0.08$	$10.16^a \pm 0.17$	$10.60^b \pm 0.23$	$13.25^a \pm 0.10$
M		$10.00^a \pm 0.03$	$11.50^a \pm 0.18$	$12.60^a \pm 0.13$									
LSD		0.10	0.62	0.43	1.14	0.95	1.01	0.17	0.65	1.70	0.15	1.03	2.14

Means not followed by the same superscript in the same column are significantly different from each other at 5% probability level.

± = Standard deviation of triplicate analysis

LSD = Least Significant difference

MM= Milk +Extract M

MN = Milk +Extract N

MO = Milk +Extract O

M = Milk only

The trend of increase in acid production with increased levels of yeast extract supplementation was observed. At 1% level of yeast extracts, N-yeast extract exhibited greater acid stimulation, while there were no significant differences between the acidity values from M and O yeast extracts (13.75% lactic acid and 13.25% lactic acid respectively).

Table 7 shows influence of various yeast extracts on the acidification of *L. casei*.

Table 7: Influence of Various Yeast Extracts on acidification of *L. casei* in skimmed milk

Concentration of yeast extract, (%)												
		0.05			0.2			0.5			1.0	
Milk Samples	Time (hrs) % lactic acid	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1
MM		10.00^a±0.18	11.75^b±0.28	15.00^b±0.15	10.10^b±0.06	12.25^a±0.10	16.25^a±0.12	10.16^a±0.01	13.50^a±0.00	17.50^a±0.01	10.15^a±0.29	14.2
MN		10.10^a±0.07	11.75^b±0.16	16.25^a±0.03	10.13^a±0.00	11.50^b±0.20	14.86^b±0.62	10.14^a±0.11	12.25^b±0.11	17.86^a±0.15	10.15^a±0.06	13.75
M0		10.15^a±0.01	10.50^c±0.23	14.67^c±0.01	10.15^a±0.07	10.50^c±0.00	12.00^c±0.04	10.16^a±0.08	13.00^a±0.05	16.25^b±0.02	10.15^a±0.10	13.50
M		10.00^a±0.00	12.50^a±0.04	15.00^b±0.29								
LSD		0.10	0.56	1.10	0.94	0.70	1.31	0.16	1.10	1.54	0.13	0.42

Means not followed by the same superscript in the same column are significantly different from each other at 5% probability level.

± = Standard deviation of triplicate analysis

LSD = Least Significant difference

MM= Milk +Extract M

MN = Milk +Extract N

MO = Milk +Extract O

M = Milk only

The results revealed that acid production increased with increase levels of all the three yeast extracts. At 0.5% yeast extract concentration, there was no significant difference in the acidity between M and N- yeast extracts at 6 hours incubation.

However at 1% extract concentration, M – yeast extract had superior values of (21.25% lactic acid) followed by N – yeast extract (19.25% lactic acid) while O–yeast extract had the least value 17.50% lactic acid.

Table 8 shows influence of various yeast extracts on the acidification of *l. acidophilus*.

Table 8: Influence of Various Yeast Extracts on acidification of *L. acidophilus* in skimmed milk

Concentration of yeast extract. (%)												
		0.05			0.2			0.5			1.0	
Milk Samples	Time (hrs) % lactic acid	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1	t_2	t_0	t_1
MM		10.00^a±0.01	11.25^b±0.55	15.00^a±0.23	10.15^a±0.19	11.25^a±0.30	15.50^b±0.07	10.15^a±0.17	10.75^b±0.01	17.00^a±0.95	10.15^a±0.01	14.50
MN		10.00^a±0.11	10.25^b±0.00	15.00^a±0.04	10.15^a±0.01	11.75^a±0.43	16.25^a±0.17	10.16^a±0.02	10.25^b±0.00	17.50^a±0.06	10.16^a±0.01	14.25
MO		10.10^a±0.37	10.00^b±0.46	11.10^c±0.02	10.15^a±0.01	11.10^a±0.07	14.50^c±0.01	10.15^a±0.12	11.50^a ±0.00	15.00^b±0.11	10.20^a±0.01	12.50
M		10.00^a±0.11	11.50^a±0.06	13.75^b±0.06								
LSD		0.11	0.63	0.12	0.91	0.73	0.66	0.17	1.10	1.97	0.15	1.53

Means not followed by the same superscript in the same column are significantly different from each other at 5% probability level.

± = Standard deviation of triplicate analysis

LSD = Least Significant difference

MM= Milk +Extract M

MN = Milk +Extract N

MO = Milk +Extract O

M = Milk only

Acidification increased with increased concentration of yeast extracts. While there was no signific difference in acidification between M and N yeast extracts at 0.5% concentration at 6 hours incubation, yeast extract had greater acidity than M-yeast extract at 6 hours incubation at 1% yeast extract level. N-ye extract had higher acid values 16.25% lactic acid than M - yeast (15.50% lactic acid) at 0.2% concentrati while O-yeast extract had the least value 14.50% lactic acid.

The results above demonstrated that different yeast extracts with their various concentrations exerted var influences on the growth and acid production of these lactic bacteria. Growth of the organisms and th acidification increased proportionally with levels of yeast extract supplementation. These results agreed w earlier works of Bracquart *at al* (1978); Olmos – Dicchara, *et al*, (1997); Amrane and Prigent, (1998) wh showed the exigency of lactic bacteria for certain amino acid and vitamins. These results also agreed w Oliveira, *et al* (2001) who showed that yeast extract supplementation improved the performance of lac bacteria in milk products.

The *Streptococci* and *Lactobacilli* are often used as mixed starter cultures in dairy industry. Apart from th health and nutritional benefits (Gilliland, 1990), they also produce various compounds such as organic aci diacetyl, hydrogen peroxide and bacteriocin or bactericidal proteins during lactic fermentations (Lindg and Dobrogosz, 1990). The antimicrobial properties of lactobacilli are of specific interest in develop strongly competitive starter cultures for food fermentation. Lactobacilli exert strong antagonistic activ against microorganisms including food spoilage organisms and pathogens by production of other inhibit substances such as bacteriocins (Brink *et al* 1994). Ogunbanwo *et al* (2003) demonstrated that yeast extr supplementation produced large quantities of bacteriocin from lactobacilli without impairing the qualities the products. Yeast extract could therefore be employed to solve fermentation problems in the milk indus without adversely affecting the quality of the product.

CONCLUSION

The stimulating effect of yeast extract on the growth and acid production of lactic bacteria could be exploi to overcome problems of "Lazy" milk, due to inhibition and proliferation of undesirable bacteria encounte frequently in the milk industry.

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