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THE EFFECT OF SOME NATURAL SWEETENERS ON THE GROWTH AND VIABILITY OF THE PROBIOTIC LACTOBACILLI IN SKIM MILK BY

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ABSTRACT

Four lactobacilli strains (*L. bulgaricus, L. gasseri, L. casei and L. acidophilus*) were cultured in buffalo skim milk containing 7% (w/v) sucrose, fructose, glucose, honey, molasses and dibis at 37°C for 24 h. The change of pH, titratable acidity and doubling time (T_d) were determined at 0, 4, 8 and 24h during fermentation. Also, their pH, titratable acidity (TA%) and viability were determined after 1, 7, 14 and 21 days of storage at 4°C. The pH values were not significantly affected by using natural sweeteners and lactobacilli strains, while the TA% was significantly ($P<0.05$) increased when grown with *L. gasseri and L.acidophilus* with higher rate than control samples after 24 h of fermentation. The mean values of doubling time (T_d) were 120.44, and 100.46 min. in the presence of molasses and glucose for *L. bulgaricus, L. acidophilus*, whereas recorded 145.70 and 110.80 min in the presence of dibis in both *L. gasseri* and *L. casei*. After 21 days of refrigerator storage, the reduction of pH and increment rate of TA% was pronounced ($P<0.05$) for *L. gasseri, L. casei* and *L. acidophilus* in all treatments as compared with control. In addition, cell viability improved by 27.21% and 14.16% for *L. bulgaricus* grown in milk containing fructose and honey, while it was 47.26 and 32.03% for *L. gasseri* in fructose and molasses, whereas it was 30.41, 36.0, 46.59 and 36.63% in the presences of molasses and dibis with grown *L. casei* and *L. acidophilus* respectively as compared to control.

It is recommended the use of the natural sweeteners as a prebiotic to enhance the growth of probiotic bacteria to achieve the beneficial health effects.

Key words: natural sweeteners, viability probiotic and lactobacilli

INTRODUCTION

Probiotic bacteria are defined as viable microorganisms that beneficially affected the host after ingestion to prevent and treat specific pathogenic conditions (Kailasapathy and Rybka, 1997 and Ziemer and Gibson, 1998). Probiotics provide several health benefits, namely improving lactose digestion, reducing side effects of antibiotics, preventing intestinal infections by production of organic acids and other antibacterial agents, balancing of intestinal bacteria, lowering of fecal enzymes, preventing cancers, lowering cholesterol and improving the immune system (Lidbeck, 1995, Spanhaak *et al.*, 1998, and Shah 2007). Lactobacilli, for instance *L.casei, L.acidophilus* and *L.gasseri,* constitute a significant proportion of probiotic lactic acid bacterium cultures used in developed countries (Vuyst,2000). *L.casei* supports already healthy immune system response, healthy cellular function and promotion of healthy bacteria in the intestinal tract (Spanhaak *et al.*, 1998, Takeshi, 2003). However, many strains of *L. casei* for their potential use in new probiotic fermented milks on the basis of their technological performances, in vitro adhesion capacity and intestinal transit tolerance after administration to rats (Bertazzoni *et al.*, 2004). *L. acidophilus* has gained prominence as an important probiotic due to its nutritional and therapeutic value (Sellars, 1991, Salji, 1992 and Goderska *et al.*, 2008).

Prebiotics are non-digestible food that beneficially affected host by selectively stimulating the growth and/or activity of limited number of bacteria in the colon (Gibson and Roberfroid, 1995, Collins and Gibson, 1999 and Gibson 2004).

Honey is a natural syrup containing primarily fructose (38.5%) and glucose (31.3%) .Other sugars in honey include maltose (7.2%) sucrose (1.5%) and various oligosaccharides (4.2%). It also contains a variety of organic acids, pyroglutamic and succnic acid (0.17 to 1.17%) (NHB, 1996). Inhibitory properties of honey against pathogens such as *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella typhi* have been demonstrated (Molan and Russell, 1989 and Molan, 1992).Recent researches suggest that it could be used as sweeteners and a good cell protective agent in fermented milk products (Chick *et al.*, 2001, Ustunol and Gandhi, 2001, Mehanna *et al.*, 2003, Zommara *et al.*, 2003, Varga, 2006 and Riazi and Ziar, 2008).

Sugar cane syrup (molasses) is a by-product of the manufacture of sugar from sugar cane. It is produced as light molasses (total sugar≥62%) for human consumption and dark molasses (total sugar≥55%) used in many food industries. Recently, molasses is used as sugar substitute in baking industry, biscuits and cakes formulas (Morano, 1976 and El-Nagar, 1997).

Dates fruits as dibis is a good source of vitamins "B1"and "B2", minerals "K, Na, Ca, Mg, Fe and Zn" and amino acids (Khalil *et al.*, 2002). The date production at less 10% is processed into a products such as frozen desserts, drink lemon beverage, flavoured laban and ice cream (Al-Juhaimi, 2003, Salama 2004 and Boeneka *et al.*, 2006).

The term symbiotic is used when a product contains both probiotics and prebiotics. A combination of probiotic and prebiotic has synergistic effects promoting growth of existing strains of beneficial bacteria in the colon as well as improving the survival, implantation and growth of newly added probiotic strains. (Ziemer and Gibson, 1998, and Schvezenmeir and deVrese 2001).The minimum therapeutic dose per day is suggested to be $10^8$to 10^9 viable cells per 100 grams of bioproduct (Shin *et al.*, 2000a,and Vuyst, 2000)

Therefore, the objective of this research was to investigate the ability of 4 lactobacilli strains to grow in skim milk supplemented with various natural sweeteners as carbohydrate source during 24 h incubation time and refrigerator storage for 21 days at 4°C.

MATERIALS AND METHODS

Materials

Fresh buffalo skim milk was obtained from Dairy Department of Faculty of Agric. Cairo Univ. Cane sugar, molasses, honey and date syrup (dibis) were purchased from local market while fructose 42 % and standard glucose were obtained from 10th Ramadan City, National Company for Maize products, Egypt. *L. gasseri* B-14168 was provided by Northern Regional Research Laboratory, Illinois USA (NRRL). *L. acidophilus*, *L. casei* and *L. bulgaricus* were obtained from Chr. Hansen's Lab., Denmark. All strains had previously been shown to posses properties required of a probiotic microorganisms including bile salt tolerance, low pH value and antagonistic activity as described by Amin *et al.* (2002). Cultures were propagated in sterile (MRS) broth using 1% inoculum and 20 h incubation at 37°C. The organism was transferred 3 time in MRS broth before used.

Experimental procedures.

Fresh buffalo skim milk was divided into 7 equal portions. The first portion was served as a control. Sucrose, fructose, glucose, honey, molasses and dibis were added individually at 7% (w/v) to the rest portions. The concentration 7% was selected according to preliminary studies.

All portions were heated at 80°C for 10 min. and cooled to room temperature. Each portion was divided into 4 equal parts and

inoculated to contain 1.5 % *L. bulgaricus, L. gasseri, L. casei* or *L. acidophilus* then incubated at 37°C for 24h. Samples were taken at 0, 4, 8 and 24h then diluted (1/10 w/v) with 0.2% (w/v) EDTA (pH 12.0) and turbidity was measured at 640 nm using Unican 8625UV/Vis Spectrometer. The uninoculated sample was diluted with 0.2% (w/v) EDTA and used as a blank according to Hughes and Hoover,1995. In a separate experiment *L. bulgaricus, L. gasseri, L. casei and L. acidophilus* were cultured at 37°C for 24h with 7% (w/v) sucrose, fructose, glucose, honey, molasses or dibis. All samples were stored at 4±1°C for 21 days.

Methods of analysis.

The pH was determined by using digital pH meter (Inolad model 720, Germany). The titratable acidity (TA%) was measured in all samples according to Ling (1963). Doubling time (T_d) for each lactobacilli strain that grown in sucrose, fructose, glucose, honey, molasses and dibis were calculated according to (Shin *et al.*, 2000a).

The viability of each lactobacilli strain was assessed at 7 days intervals by using the pour plate with lactobacilli MRS agar. The plates were overlayed with the same medium and incubated at 37°C for 48h according to Vanderzant and Splittstoesser, 1992. The colonies were counted using a Quebec colony counter (Fisher Scientific, Pittsburg, Pa. USA). Colony-forming units (cfu/ml) were counted and the results expressed as their $\log_{10}$ values.

Statistical analysis was conducted using ANOVA analysis (System User's Guide SAS 1997).

The means ($P< 0.05$) were carried out by using Duncan multiple range tests. All analysis were done in duplicate.

RESULTS AND DISCUSSION

1- Changes in pH values, titratable acidity(TA%) and doubling time (T_d) during fermentation.

Changes in pH values of skim milk containing 7% of each natural sweeteners and lactobacilli strains are presented in Table (1). The obtained results indicated that the reduction of pH value was at the rate of 46.48, 43.71 and 43.41% in case of fructose in strains of *L.bulgaricus, L. gasseri and L. casei* as compared with control and other sweeteners after 24h fermentation time at 37°C.

In case of *L. acidophilus* the reduction of pH-values were not affected by the type of sweeteners. The mean pH value was the highest in milk containing glucose with *L. acidophilus* (5.81) but they were the lowest in the presence of molasses with *L.gasseri* (4.85) .

Table (2) shows the changes in the TA% as affected by the type of sweeteners. The supplementation of milk with sweeteners caused a significant increase in the TA% by all lactobacilli at all time interval as compared with control. The variations in the TA% may be attributed to the different metabolites of the used strains and the composition of the sweeteners. These results are confirmed by Shin *et al.* (2000,b) and Ustunol and Gandhi (2001).

On contrary of the present results, Riazi and Ziar (2008) reported that lactic acid production was not influenced by sweetener type.

Data presented in Table (3) show the doubling time(T_d) for *L. bulgaricus L. gasseri, L. casei and L. acidophilus*/gram as affected by the different sweeteners at 4h intervals, up to 24hs of incubation. Doubling time was used as a measure of the efficacy of the sweeteners in modulating growth rate. The doubling time (T_d) of *L. bulgaricus* grown with different sweeteners decreased as compared with the control. Among the tested sweeteners sucrose, honey and molasses were the most effective in enhancing growth rate of *L. bulgaricus* after 4 and 8h fermentation as evidenced by shorter doubling times. However, among the sweeteners tested honey and dibis were most effective in enhancing growth rate of *L. gasseri* whereas, it was glucose and dibis with

grown *L. acidophilus* .In case of *L. casei* the doubling time (T_d) was markedly increased ($P<0.05$) in all treatments except dibis was similar to control.

The data in Table (3) indicate that glucose, honey molasses and dibis are the preferred sweeteners by lactic acid bacteria owing to the presence of various carbohydrates (oligosaccharides) present in these sweeteners. While Ustunol and Gandhi (2001) and Mehanna *et al.* (2003) reported that the honey was the preferred sweetener by bifidobacteria compared to sucrose, fructose and glucose. Desai *et al.* (2004) observed that the Hi-maize as, a prebiotic, stimulate the growth of lactobacilli strains *L. casei, L. paracasei*, and *L.rhamnosus* in RMS with doubling time of 301min. The mean value of doubling time (T_d) was decreased at percentage rate of 63.15, 20.21, 53.55 and 2.22% when *L. bulgaricus, L. gasseri*, *L. acidophilus* and *L.casei* are grown in the presence of molasses, dibis, glucose and dibis respectively.

2-Changes in pH values, titratable acidity(TA%) and viability of lactobacilli strains in fermented skim milk during storage at 4°C

Data presented in Fig (1) show that the pH values of all treatments were decreased ($P<0.05$). At the beginning of storage, the pH values in fermented milk with *L. bulgaricus* and *L. casei* ranged from 3.5- 3.91 and 3.66 - 3.88 whereas it was 3.59 – 4.93 and 4.6 – 4.91 with *L. gasseri* and *L. acidophilus* respectively.

When *L. gasseri* is grown in skim milk, pH values had similar trend to *L. acidophilus* which is in accordance with Zhu and WU (2000) who reported that *L. acidophilus* is related to lactobacilli species such as *L. gasseri* and *L. johanosusi*

At the end of cold storage, the lowest pH value was recorded by *L.bulgaricus* in the presence of fructose followed by *L.casi* in the presence of honey. The change of pH values in sucrose and molasses in case of *L. acidophilus* were similar to control at all time intervals.

In the case of *L. gasseri*, the pH values of all treatments were decreased more than control except samples containing dibis after 21days of refrigerator storage . Also, the rate of pH reduction in all treatments were higher than control for *L. acidophilus*.

Statistical analysis, show that the effect of sweeteners on the mean pH ranked as follows: molasses > control > honey > sucrose > dibis >glucose > fructose.

Fig. (2) shows the changes of TA% during refrigerator storage of lactobacilli strains as affected by sweeteners type. It revealed that TA% of control samples with *L. bulgaricus* and *L. casei* is higher than samples containing sweeteners, whereas it was lower in case of *L. gasseri* and *L. acidophilus*. By the end of storage, samples containing molasses and dibis recorded lower TA% values by *L. gasseri* and *L. acidophilus* than other sweeteners and control. In the case of *L. bulgaricus* the reduction in TA% was much higher in both molasses and dibis compared with control and other sweeteners. In case of *L. casei* the samples containing dibis, the TA% decreased along the storage periods. These results may due to the low temperature and low pH which inhibit enhancing the growth of the cultures.

The viability of *L. bulgaricus* after 14 days of refrigerated at 4°C in samples containing sucrose is the highest (Fig 3). By the end of storage, the count of *L. bulgaricus* was significantly decreased in all treatments except samples containing dibis. This decrease in the viability may be attributed to the reduction of pH value, secretion of antimicrobial substances and accumulation of bacterial metabolites in the culture products during cold storage (Lankaputhra *et al.*, 1996, Dave and Shah, 1997). Riazi and Ziar (2008) reported that lactic acid bacteria (LAB) growth was slightly inhibited when 10% honey was added and yoghurt acidity was moderate.

As the storage progressed, the viability of *L. gasseri* was simulated in all treatments with different rates. These results indicated that *L. gasseri* can survive well at low temperature.

Table (1):The pH value of skim milk containing natural sweeteners and Lactobacilli strains.

lactobacilli strains	Incubation Time(h)	Control	Sucrose	Fructose	Glucose	Honey	Molasses	Dibis	Mean
Lb	**0**	6.35	6.37	6.54	6.30	6.22	6.13	6.08	**6.28^{a}**
	4	6.04	5.97	5.92	6.20	5.78	5.69	5.61	**5.84^{b}**
	8	5.25	5.24	5.19	5.14	5.17	5.25	5.29	**5.22^{c}**
	24	3.62	3.79	3.50	3.69	3.72	3.90	3.91	**3.71^{f}**
	Mean	**5.32**	**5.34**	**5.29**	**5.25**	**5.22**	**5.19**	**5.22**	
Lg	**0**	6.60	6.52	6.52	6.53	6.40	6.04	6.20	**6.43^{a}**
	4	6.07	5.99	5.88	5.79	5.22	5.30	5.55	**5.71^{b}**
	8	5.58	5.58	5.36	5.25	5.11	4.42	5.11	**5.28^{c}**
	24	4.61	3.96	3.67	4.74	4.77	3.53	4.93	**4.56^{d}**
	Mean	**5.71**	**5.68**	**5.68**	**5.57**	**5.22**	**4.85**	**5.45**	
Lc	**0**	6.47	6.46	6.45	6.35	6.35	6.27	6.15	**6.35^{a}**
	4	5.96	5.76	5.94	5.84	5.84	5.73	5.67	**5.82^{b}**
	8	5.15	5.16	5.17	4.97	5.13	5.25	5.14	**5.13^{c}**
	24	3.75	3.76	3.65	3.88	3.72	3.82	3.83	**3.77^{f}**
	Mean	**5.31**	**5.34**	**5.30**	**5.34**	**5.26**	**5.27**	**5.13**	
La	**0**	6.55	6.52	6.56	6.59	6.46	6.36	6.28	**6.47^{a}**
	4	6.06	6.07	5.95	6.05	5.89	5.83	5.84	**5.96^{b}**
	8	5.94	5.81	5.84	5.18	5.63	5.67	5.54	**5.74^{b}**
	24	4.79	4.79	4.87	4.62	4.79	4.65	4.91	**4.76^{d}**
	Mean	**5.77**	**5.70**	**5.79**	**5.81**	**5.69**	**5.63**	**5.65**	
General mean		**5.49**	**5.45**	**5.42**	**5.42**	**5.35**	**5.29**	**5.31**	

Different superscript (a,b,c....) at the same column and row are significantly different (P<0.05).

Lb : *L.bulgaricus bulgaricus* **Lc** : L. casei
Lg : *L. gasseri* ***La*** : *L. acidophilus*

The viability of *L. casei* and *L. acidophilus* were almost similar. Their counts are gradually increased in all treatments to reach its maximum after 14 days then declined at the end of storage period which is in accordance with Sharaf *et al.*, 2003. The reduction of viability may be due to the presence of acidic metabolites which inhibit the growth of *L. casei* and *L. acidophilus* (Lankaputhra, *et al.*, 1996, and Liong and Shah, 2005). Dave and Shah (1997) noticed a higher production of hydrogen peroxide with the ABT culture in yoghurt which caused a partial injury to the cell of *L. acidophilus.*

The highest viability of *L. casei* and *L. acidophilus* by the incorporation of molasses were recorded at 14 days of storage. Morano, 1976, Chick *et al.*, 2001 and Khalil *et al.*, 2002 suggests that both organisms have a higher requirement for one or more compounds from honey, molasses and dibis such oligosaccharides and organic acids.

Statistical analysis, revealed that the growth of lactobacilli strains supplemented with carbohydrate sources at 7% level were ranked as follows: *L.casei* > *L. gasseri* > *L. acidophilus* > *L. bulgaricus*. Moreover, the growth of lactobacilli strains was higher in case of dibis than other sweeteners and control samples.

Generally, numbers of all lactobacilli strains remained more than 10^8 cfu/ml in all treatments until the end of storage. These counts are higher than 10^7 cfu/ml, which is the level suggested by some authors to have health promoting effect and good way to overcome the reduction in the counts of lactobacilli bacteria in the functional fermented milks. These results are in agreement with Nighswonger *et al.* (1996)and Roushdy *et al.* (1996).

It could be concluded that there is a synergistic effect between carbohydrate components in enhancing lactobacilli growth and viability.

Table (2): The titratable acidity (TA%)of skim milk containing natural sweeteners and lactobacilli strains.

lactobacilli strains	Incubation Time(h)	Control	Sucrose	Fructose	Glucose	Honey	Molasses	Dibis	Mean
Lb	0	0.235^{V-Z}	0.240^{V-Z}	0.240^{V-Z}	0.245^{V-Z}	0.263^{U-Z}	0.320^{S-Z}	0.330^{P-Z}	**0.267^{g}**
	4	0.365^{N-Z}	0.380^{L-Z}	0.380^{L-Z}	0.375^{M-Z}	0.395^{K-Z}	0.360^{O-Z}	0.405^{K-Z}	**0.380^{f}**
	8	0.580^{G-P}	0.570^{G-Q}	0.445^{J-X}	0.520^{G-T}	0.505^{G-T}	0.458^{I-W}	0.470^{H-V}	**0.506^{e}**
	24	1.187^{A}	1.095^{AB}	0.930^{BCD}	1.01^{A-D}	1.130^{AB}	1.060^{ABC}	1.030^{A-C}	**1.063^{a}**
	Mean	**0.591^{a-d}**	**0.570^{a-d}**	**0.498^{d-f}**	**0.537^{bcd}**	**0.573^{d-f}**	**0.544^{bcd}**	**0.605**	
Lg	0	0.225^{W-Z}	0.233^{W-Z}	0.180^{Z}	0.182^{Z}	0.195^{YZ}	0.264^{U-Z}	0.365^{N-Z}	**0.234^{g}**
	4	0.335^{Q-Z}	0.310^{T-Z}	0.360^{Q-Z}	0.411^{K-Z}	0.410^{K-Z}	0.510^{G-T}	0.540^{G-T}	**0.411^{f}**
	8	0.410^{K-Z}	0.400^{K-Z}	0.510^{G-T}	0.420^{K-Y}	0.470^{H-V}	0.613^{F-L}	0.675^{G-J}	**0.499^{e}**
	24	0.564^{G-R}	0.715^{EFG}	0.540^{G-T}	0.63^{F-K}	0.63^{F-K}	0.687^{E-I}	0.720^{EFG}	**0.640^{d}**
	Mean	**0.383^{l-m}**	**0.414^{fi}**	**0.397^{ghi}**	**0.410^{bcd}**	**0.426^{bcd}**	**0.518^{c-f}**	**0.575^{a-d}**	
Lc	0	0.225^{W-Z}	0.222^{G-U}	0.240^{V-Z}	0.250^{V-Z}	0.260^{U-F}	0.310^{G-U}	0.335^{Q-Z}	**0.263^{g}**
	4	0.372^{M-Z}	0.365^{N-Z}	0.360^{O-Z}	0.374^{M-Z}	0.405^{K-Z}	0.490^{G-U}	0.493^{G-U}	**0.408^{f}**
	8	0.700^{E-G}	0.630^{F-K}	0.573^{CDE}	0.570^{G-Q}	0.550^{G-S}	0.570^{G-Q}	0.600^{F-M}	**0.642^{d}**
	24	1.20^{A}	0.980^{A-D}	1.00^{A-D}	0.940^{BCD}	1.00^{A-D}	1.120^{AB}	0.960^{BCD}	**1.028^{b}**
	Mean	**0.623^{abc}**	**0.548^{ab}**	**0.543^{abc}**	**0.533^{b-e}**	**0.550^{bcd}**	**0.622^{a}**	**0.597^{a-d}**	
La	0	0.220^{XYZ}	0.220^{XYZ}	0.220^{XYZ}	0.230^{W-Z}	0.240^{V-Z}	0.260^{U-Z}	0.260^{U-Z}	**0.235^{g}**
	4	0.345^{P-Z}	0.326^{S-Z}	0.546^{GT}	0.330^{R-Z}	0.400^{K-Z}	0.487^{G-U}	0.535^{G-T}	**0.424^{f}**
	8	0.370^{N-Z}	0.530^{G-Z}	0.566^{G-R}	0.547^{G-SZ}	0.410^{K-Z}	0.630^{F-K}	0.610^{F-L}	**0.523^{e}**
	24	0.450^{J-X}	0.630^{F-K}	0.595^{F-O}	0.566^{K-U}	0.666^{E-J}	0.820^{DEF}	0.680^{DEF}	**0.629^{d}**
	Mean	0346^{i}	0.426^{e-i}	0.484^{d-g}	0.413^{e-i}	0.430^{e-i}	0.549^{bcd}	0.521^{b-f}	
General mean		**0.346^{l}**	**0.426^{e-i}**	**0.484^{d-g}**	**0.413^{e-i}**	**0.430^{e-h}**	**0.549^{bcd}**	**0.521^{b-fa}**	

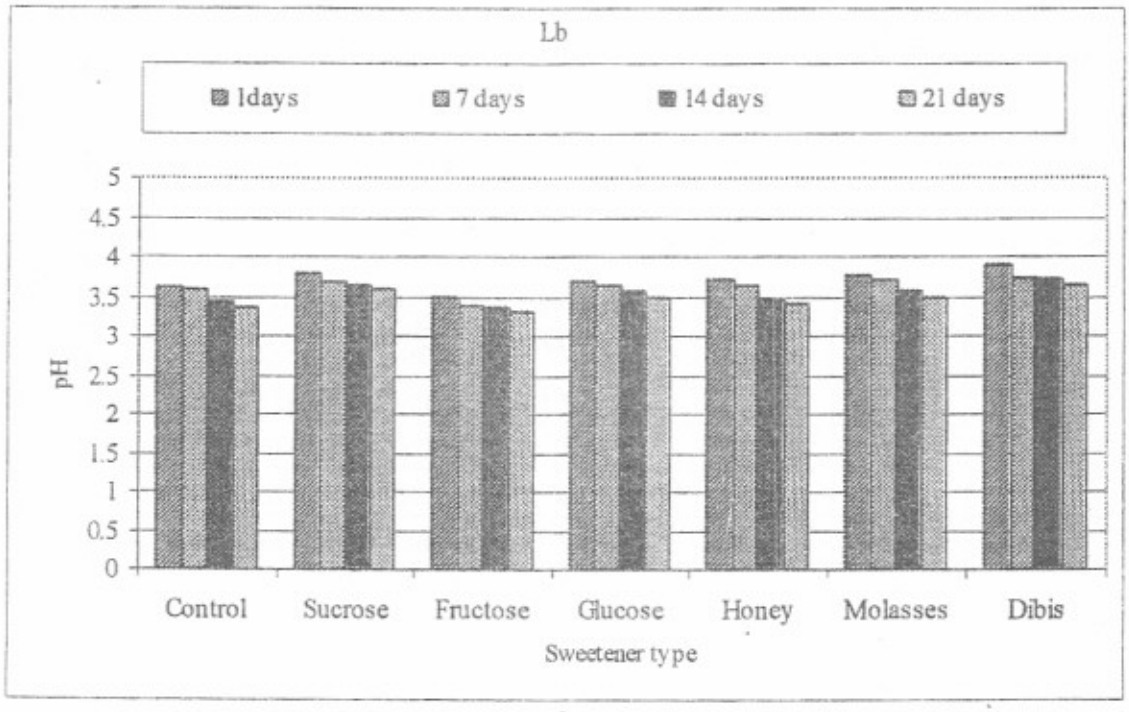
Different superscript (A,B,C..., a,b,c) at the same column and row are significantly different ($P<0.05$).

Table (3): Doubling time (T_d) of lactobacilli strains as affected by the natural sweeteners.

Sweetener type	Incubation Time(h)	*Lb*	*Lg*	Lc	*La*	Mean
Control	4	261.77^{E-N}	114.48^{U-Y}	48.93^{Y}	118.55^{R-Y}	**135.94^{f-i}**
	8	277.76^{E-L}	147.17^{N-Y}	75.52^{V-Y}	171.57^{L-X}	**166.37^{e-g}**
	24	441.08^{BCD}	291.91^{E-K}	183.89^{P-Q}	426.35^{BCD}	**335.81^{bc}**
	Mean	**326.87^{b}**	**182.61^{d-h}**	**102.75^{j}**	**238.58^{dc}**	
Sucrose	4	81.98^{V-Y}	240.19^{F-Q}	126.70^{P-Y}	126.55^{P-Y}	**145.12^{F-I}**
	8	92.13^{U-Y}	345.57^{C-F}	110.02^{T-Y}	192.74^{I-U}	**185.94^{def}**
	24	210.31^{I-S}	425.73^{BCD}	242.79^{F-N}	345.89^{C-F}	**306.18^{c}**
	Mean	**128.14^{h-j}**	**337.16^{b}**	**162.62^{e-j}**	**221.73^{cde}**	
Fructose	4	155.11^{M-Y}	233.48^{F-S}	136.68^{P-Y}	139.46^{O-Y}	**166.18^{d-g}**
	8	184.04^{J-U}	257.23^{E-O}	218.65^{F-S}	١٧٤,٠٣$^{L-X}$	**208.48^{de}**
	24	347.56^{C-F}	600.50^{A}	333.65^{DGY}	203.25^{I-U}	**401.13^{a}**
	Mean	228.90^{cd}	363.65^{ab}	229.66^{bc}	182.54^{d-h}	
Glucose	4	131.42^{P-Y}	351.55^{D-F}	174.15^{K-W}	63.31^{X-Y}	**177.50^{d-g}**
	8	436.16^{BCD}	370.66^{CDE}	181.57^{J-U}	100.71^{T-Y}	**296.04^{c}**
	24	605.12^{A}	420.59^{CBD}	304.60^{E-I}	170.84^{L-X}	**374.67^{ab}**
	Mean	**422.58^{a}**	**377.45^{ab}**	**220.11^{cde}**	**110.80^{ji}**	
Honey	4	103.61^{T-Y}	112.46^{T-Y}	173.03^{L-X}	336.31^{EDF}	**180.99^{def}**
	8	168.38^{M-W}	139.23^{O-Y}	234.33^{F-R}	369.69^{CDE}	**225.61^{d}**
	24	203.88^{I-U}	269.71^{E-M}	272.89^{E-L}	454.88^{BC}	**300.34^{c}**
	Mean	**155.56^{e-j}**	**173.80^{d-i}**	**155.56^{i-n}**	**386.96^{ab}**	
Molasses	4	58.31^{XY}	147.60^{N-Y}	130.31^{P-Y}	83.81^{V-Y}	**105.01^{IJ}**
	8	101.23^{T-Y}	186.78^{J-U}	218.65^{I-S}	122.13^{Q-Y}	**155.82^{e-g}**
	24	201.78^{I-U}	483.13^{B}	307.83^{E-H}	292.72^{E-J}	**321.36^{c}**
	Mean	**120.44^{hji}**	**272.50^{c}**	**226.26^{cde}**	**166.22^{d-g}**	
Dibis	4	147.16^{N-Y}	60.82^{XY}	55.71^{XY}	110.62^{T-Y}	**93.58^{i}**
	8	159.21^{M-Y}	114.54^{U-Y}	75.06^{W-Z}	140.73^{O-Y}	**122.24^{hi}**
	24	257.55^{E-O}	261.74^{E-N}	189.51^{I-U}	204.88^{I-U}	**228.29^{d}**
	Mean	187.78^{d-g}	145.70^{h-i}	100.46^{j}	151.90^{h-i}	
General mean		**224.33^{B}**	**264.70^{a}**	**180.75^{c}**	**208.39^{b}**	

Different superscript (A,B,C..., a,b,c) at the same column and row are significantly different ($P<0.05$)..

a



b

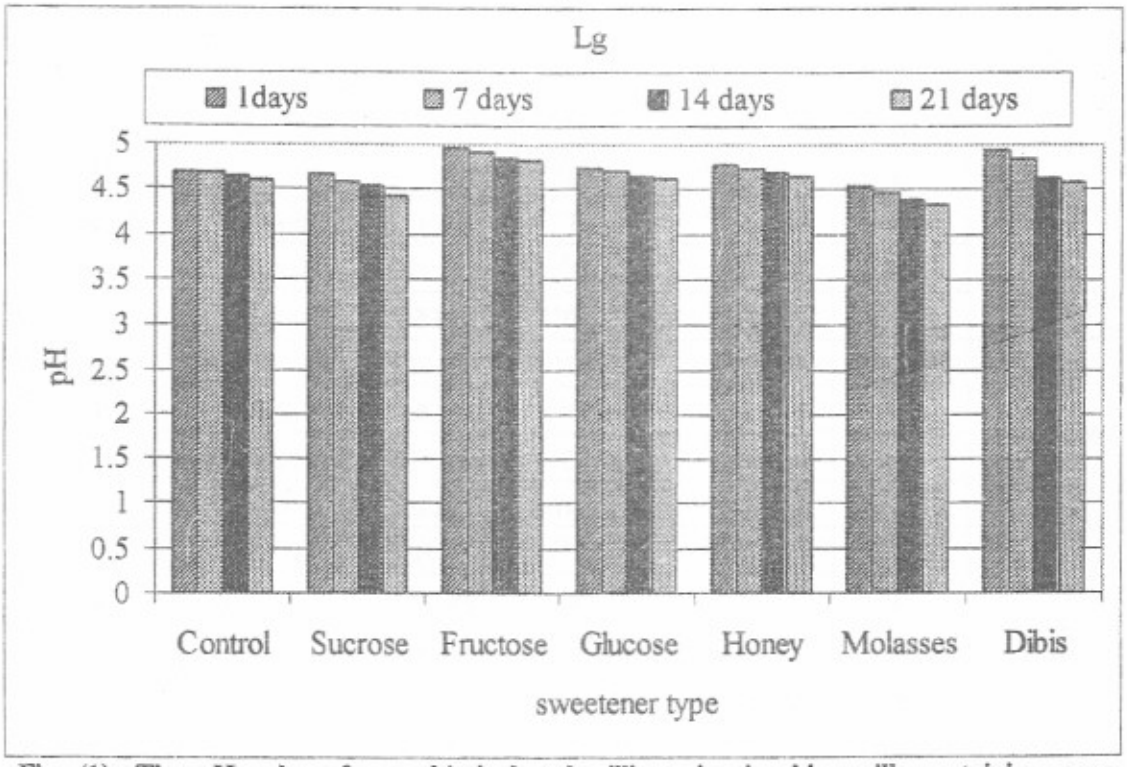
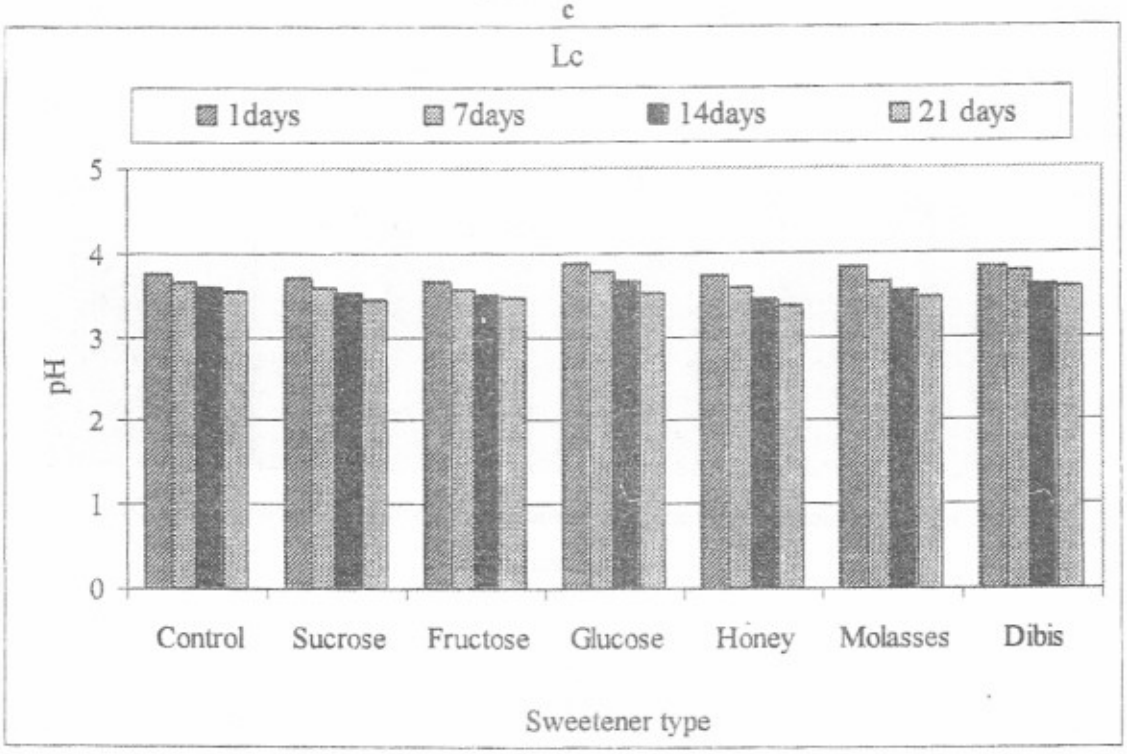


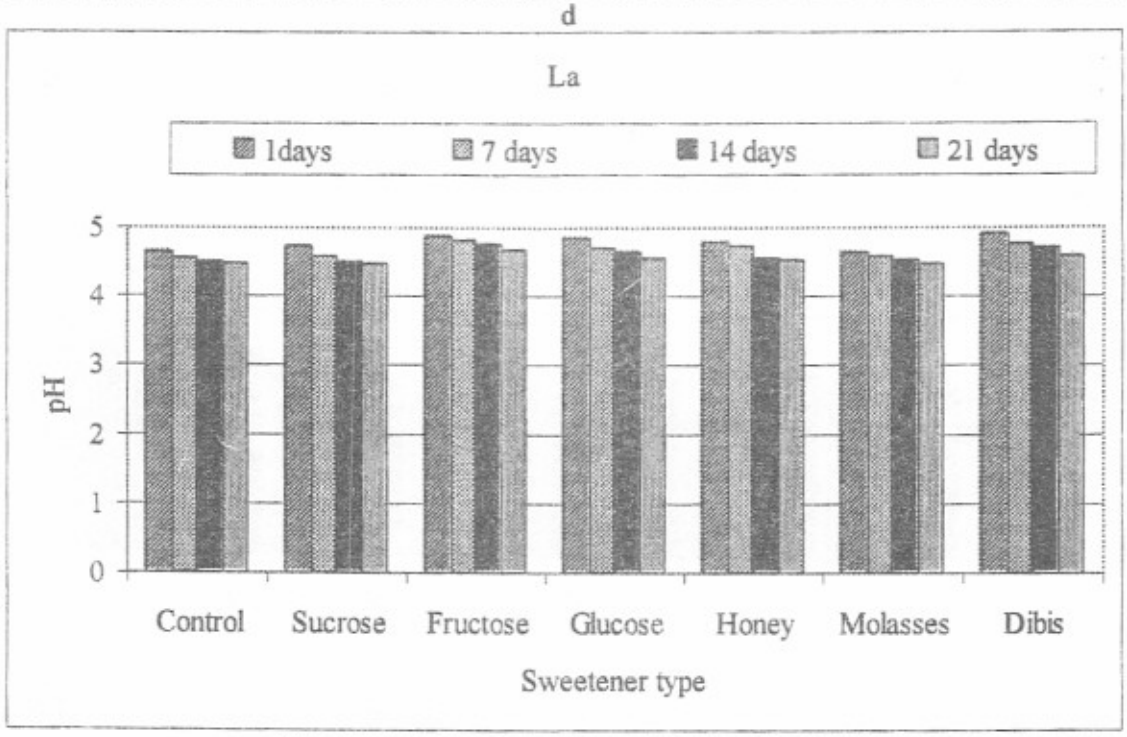
Fig. (1): The pH values for probiotic lactobacilli strains in skim milk containing some natural sweeteners.
(a) *L. bulgaricus* (b) *L.gasseri*

Fig. (1): Continued

c

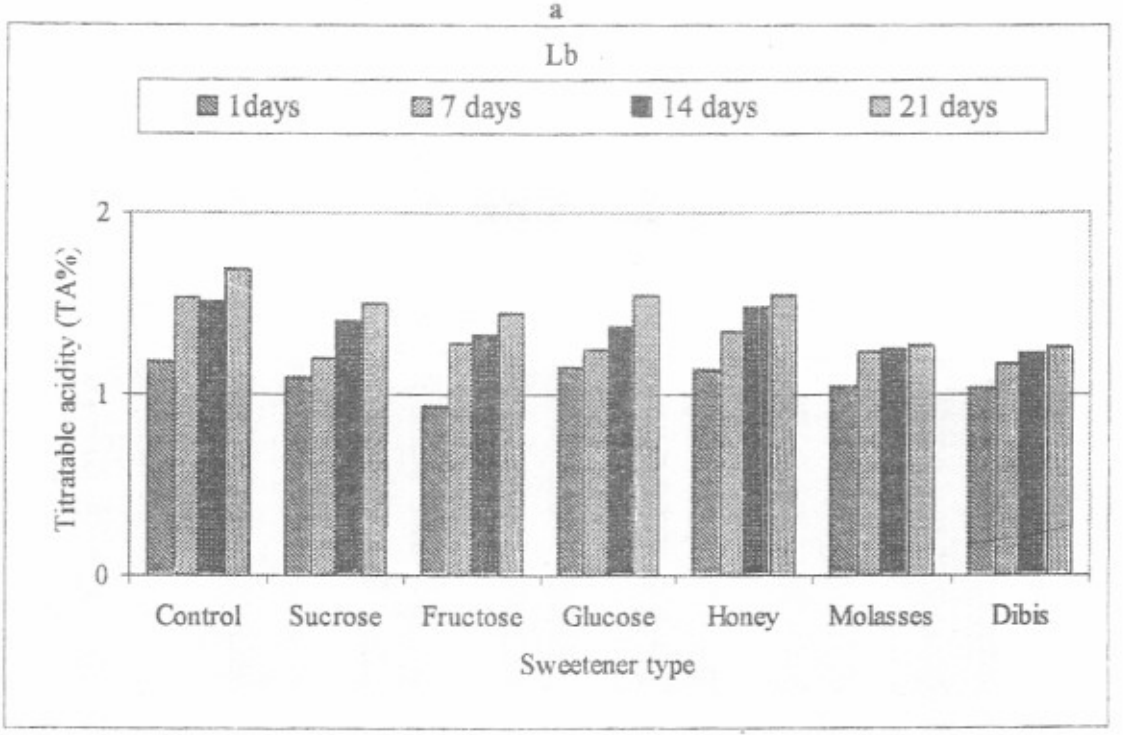


d



(c) *L.casei* (d) *L.acidophilus*

a



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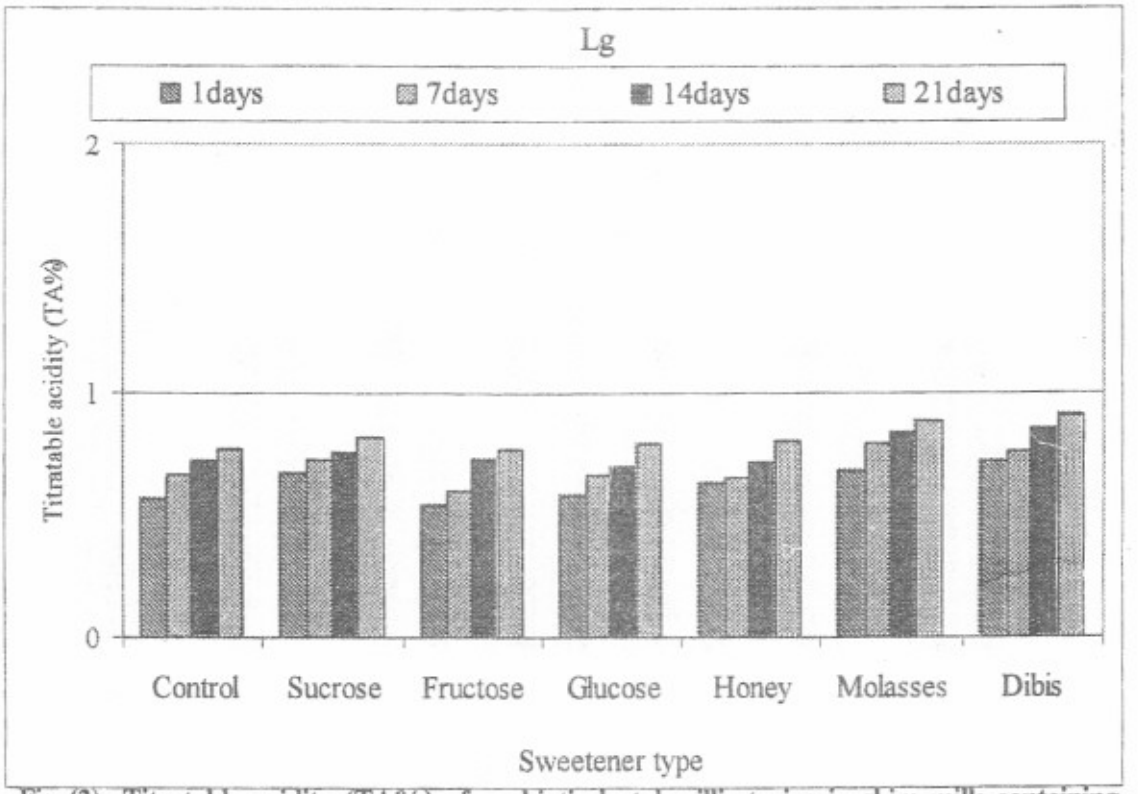
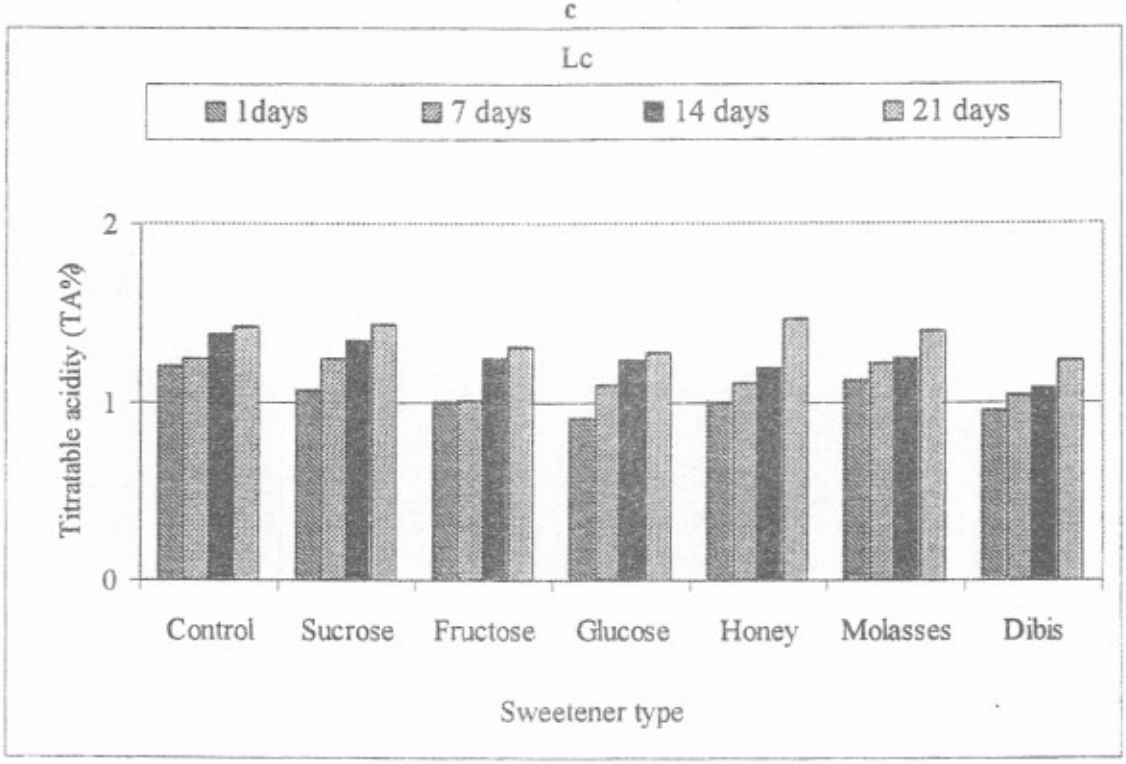


Fig (2): Titratable acidity (TA%) of probiotic lactobacilli strains in skim milk containing some sweeteners.

(a) *L. bulgaricus* (b) *L. gasseri*

Fig. (2): Continued

c



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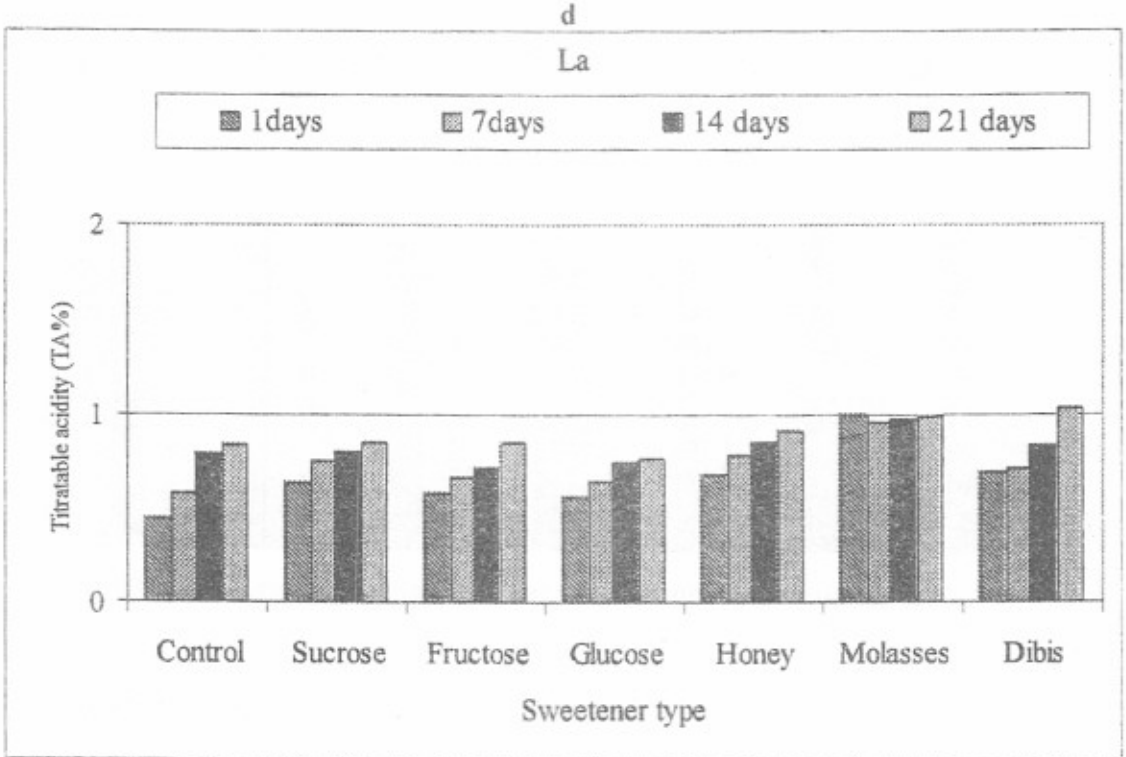


(c) *L.casei* (d) *L.acidophilus*

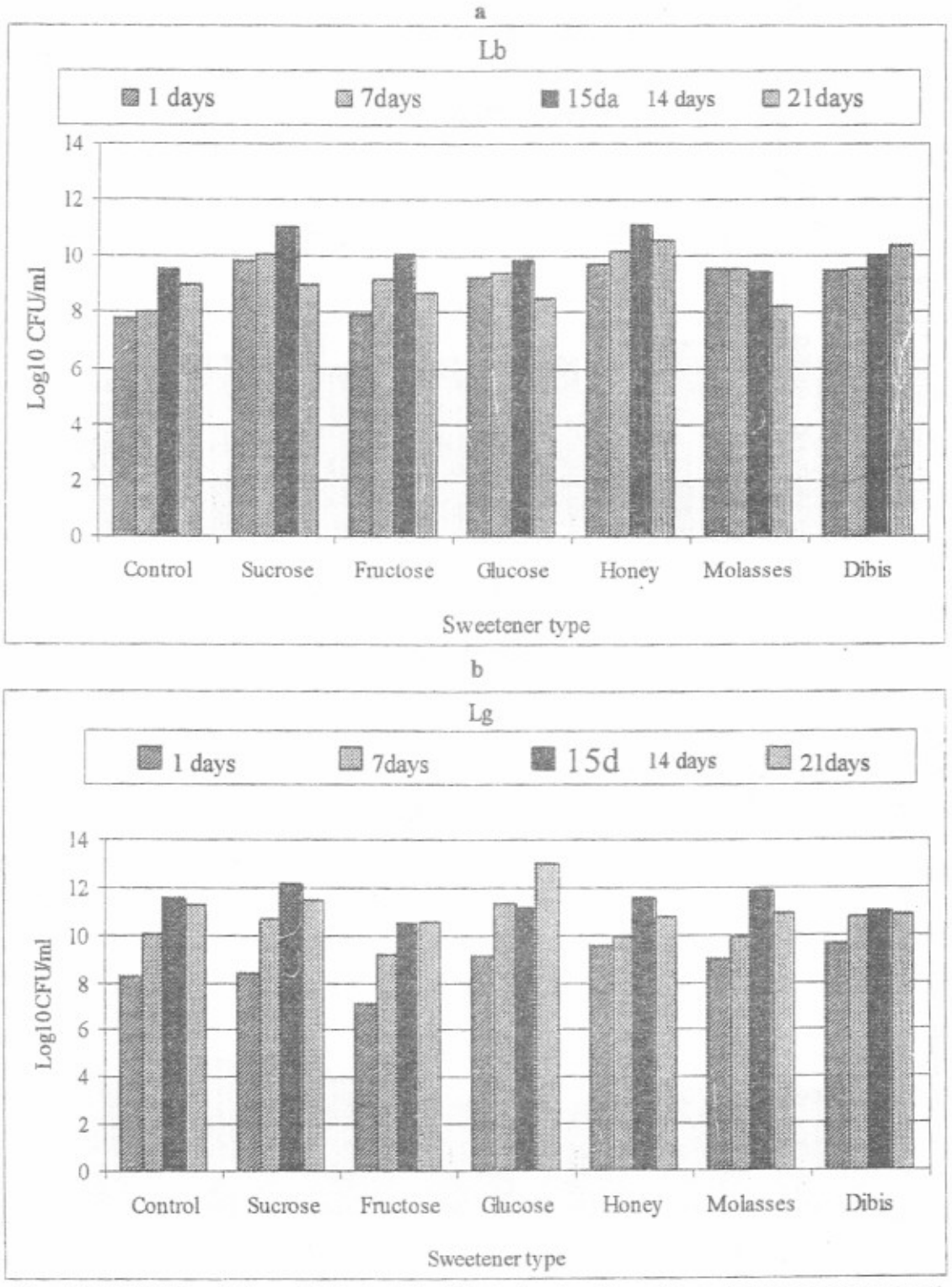
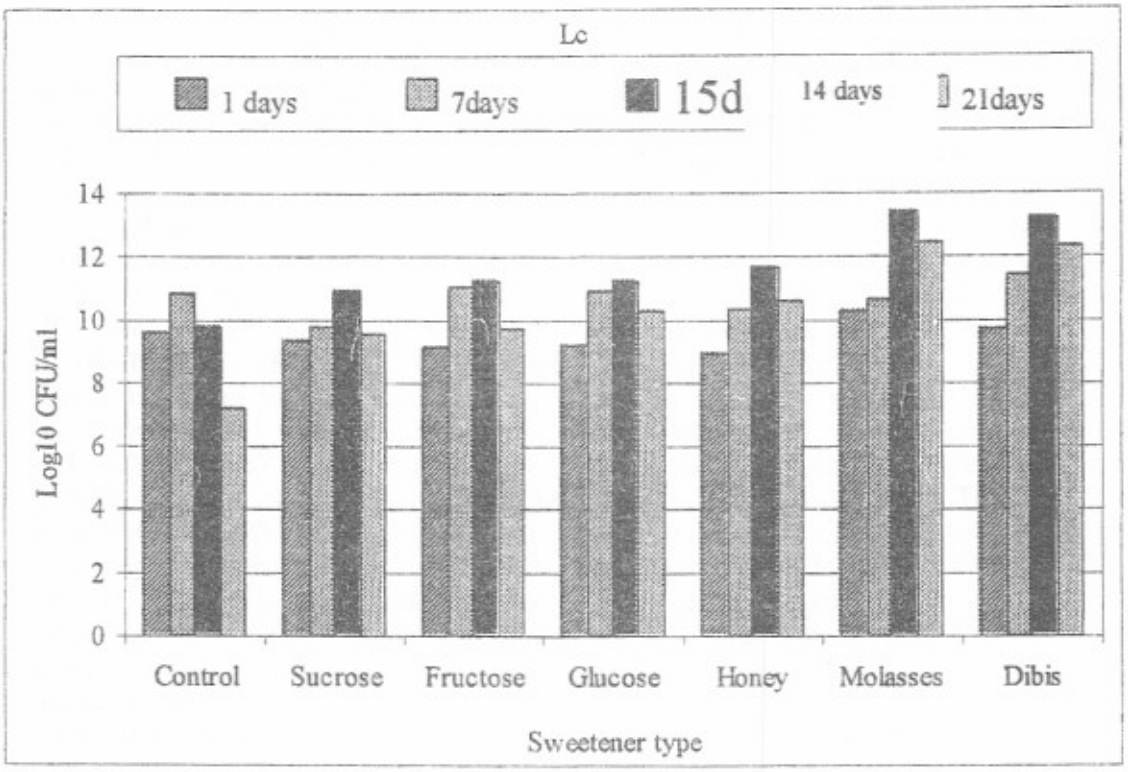


Fig. (3): Viability of 4 probiotic lactobacilli strains in skim milk containing some natural sweeteners.

(a) *L.bulgaricus* **(b) *L.gasseri***

Fig. (3): Continued

C



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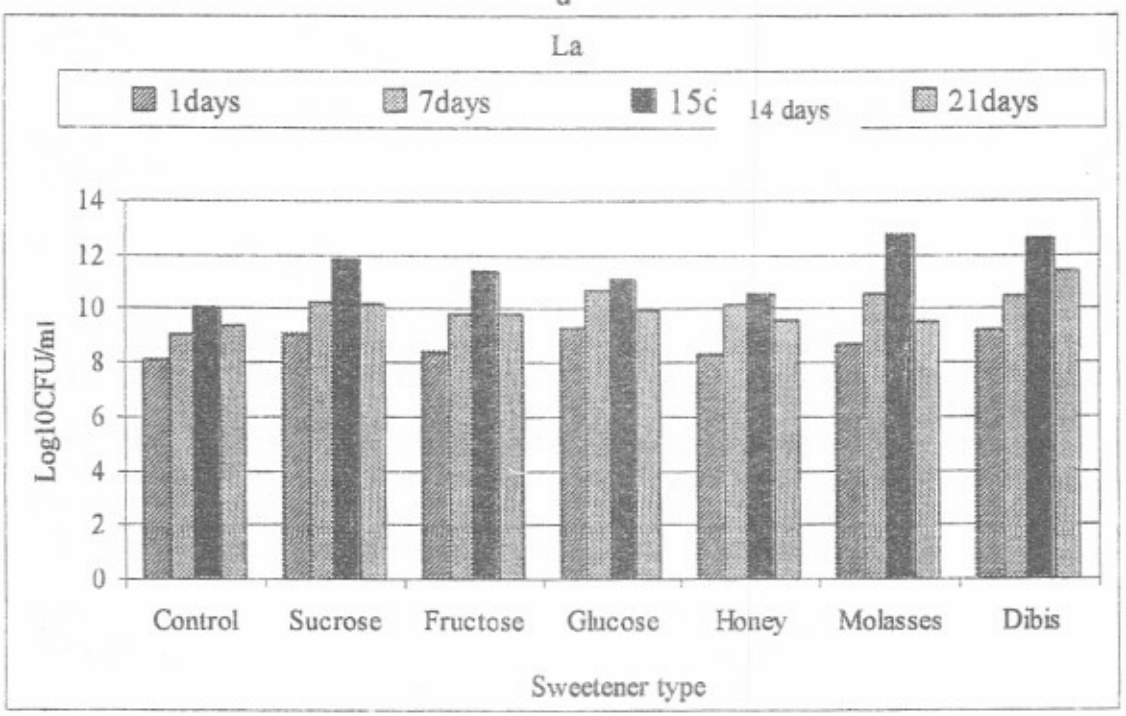

(c) *L. casei* (d) *L.acidophilus*

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تأثير بعض السكريات الطبيعية علي نمو وحيوية بكتريا الـ Lactobacilli الداعمة للحيوية في اللبن الفرز

فاطمة على متولي رمضان
قسم علوم وتكنولوجيا الألبان – كلية الزراعة – جامعة القاهرة – الجيزة – مصر

استخدم في هذا البحث ٤ سلالات من ال Lactobacilli (*L. bulgaricus, L. gasseri, L. casei, L. acidophilus*) ولقحت في اللبن الفرز الجاموسى المحتوى على السكريات الطبيعية(السكروز والفركتوز والجلوكوز والعسل الأبيض والمولاس والدبس)بنسبة ٧% وتم تحضين العينات على ٣٧°م ودرس التغيرات في قيم ال pH و% الحموضة ومعدل النمو وتضاعف الأعداد البكتيرية (T_d) على فترات صفر ، ٤ ، ٨ ، ٢٤ ساعة ثم خزنت تلك العينات في الثلاجة على٤°م وتم تتبع التغيرات فى قيم ال pH و% للحموضة والأعداد البكتيرية الحية $\log_{10}$cfu/ml خلال فترات التخزين ١ ،٧ ، ١٤ ، ٢١ يوماً، وأوضحت النتائج بعد التحليل الاحصائى ما يلي:

١- إضافة السكريات ليس لها تاثيرمعنوى على متوسط انخفاض قيم ال pH في وجود كل بكتريا البروبيونيك الداعمة الحيوية

٢- إضافة السكريات لها تأثير معنوي على زيادة % للحموضة في العينات المحتوية على *L. gasseri* ، *L. casei* ، *L. acidophilus,* بالمقارنة بالكونترول بعد التخزين لمدة ٢١يوم في الثلاجة

٣- حدوث أعلى انخفاض في متوسط تضاعف الأعداد (T_d) doubling time وكانت ١٢٠,٤٤ دقيقة عند نمو *L. bulgaricus* فى وجود المولاس ١٠٠,٤٦ دقيقة عند نمو *L. acidophilus* فى وجود الجلوكوز بينما تصل فى وجود الدبس إلى ١٤٥,٧٠ دقيقة، ١١٠,٨٠ دقيقة عند نمو كلا من *L. gasseri* ، *L. casei* على التوالي.

٤- لوحظت زيادة فى معدل انخفاض قيم ال pH وارتفاع الحموضة في العينات المخزنة لمدة ٢١ يوم على ٤°م عند نمو *L. gasseri* ، *L.casei* ، *L. acidophilus* بالمقارنة بالكونترول.

٥- حدثت زيادة فى نمو أعداد البكتريا الحيوية بمعدل ٢٧,٢١% ، ١٤,١٦% بنمو *L.bulgaricus* فى اللبن المحتوى على الفركتوز والعسل الأبيض ونسبة ٤٧,٢٦% ، ٣٢,٠٣% بنمو *L. gasseri* فى كل من الفركتوز والمولاس بينما تصل إلى ٣٠,٤١% ، ٣٦,٠٠% ، ٤٦,٥٩% ، ٣٦,٦٣% فى وجود المولاس والدبس لكل من *L. casei* ، *L. acidophilus* على التوالى بعد التخزين للعينات لمدة ١٤ يوم على 4°م بالمقارنة بالكونترول

يوصي هذا البحث باستخدام هذه السكريات الطبيعية وإضافتها للأغذية كمواد Prebiotic لزيادة أعداد بكتريا البروبيوتيك الداعمة للحيوية في اللبن الفرز ليكسبة الصفات الصحية المطلوبة فى الأغذية الوظيفية.