Al-Azhar J. Agric. Res., Vol. 39
PP. 37-46, (June), 2004

STUDIES ON BIFIDUS-MILK

Noeman, A. A. and Y. A. Mohamed,

Dairy Depart., Fac. of Agriculture, Al-Azhar University Cairo, Egypt

ABSTRACT: Bifidus-milk was made from partially skimmed buffaloe's milk (3.0 + 0.1% fat) and cultured with either 10% of Bif. bifidum or Bif. longum in order to choice the proper starter culture. The mostly best properties exerted Bifidus-milk made with Bif. bifidum through its higher total solids, fat, total nitrogen, total volatile fatty acids (TVFA) and diacetyl contents. Moreover, from the rheological point of view, the most acceptable Bifidus-milk through its lower synersis, higher firmness and viscosity against that made with Bif. longum.

Furthermore, results of the organoleptic scoring and nutritional evaluation of the resultant Bifidus-milk confirmed the previous results.

In-order to reduce the production time of Bifidus-milk, 0.3% of Glucono-Delta-Lactone (GDL) was used incorporation with 5% of Bif. bifidum. It was interest to notice that using Bif. bifidum in association with GDL led to reduce the coagulation time by 42.9%.

However, sensory evaluation, rheological assessment and nutritional values of resultant Bifidus-milk showed slight decreases.

INTRODUCTION

Bifidus is a fermented milk product, based on using the culture of Bif. bifidum or Bif. longum. This products was reported to have an excellent nutritional and therapeutic values (Badawi and El-Sonbaty, 1997; Misra and Kuila, 1992 and 1994; Sarkar and Misra, 1998).

The use of Bifidobacterium cultures in dairy industry is one of the most promising application of starter cultures in dairy processing. The use of Bif. longum or Bif. bifidum for the production of Bifidus-milk was suggested by many investigators.

In order to choice the proper starter, the two species of bifidobacteria were used in the manufacture of bifidus-milk.

On the other hand, the relatively slow acid production in milk bifidobacteria and extending the production time of bifidus-milk became one of the serious problems facing this industry. In order to avoid this problem Glucono-Delta-Lactone (GDL) was used as an acidogen.

However, this work aimed to choice the proper starter for the manufacturing of Bifidus-milk and also to investigate the effect of using GDL incorporation with Bif. longum or Bif. bifidum on the coagulation time and different properties of resultant Bifidus-milk.

MATERIALS AND METHODS

Materials:

Starter cultures:

Bifidobacterium bifidum ATCC-15696 and Bifidobacterium longum ATCC-15708, were secured from Cairo Microbiological Resources Center (Cairo MIR-CEN),Cairo, Egypt .

Starter cultures were propagated daily in 10 % skimmilk autoclaved at 121oC for 15 min., maintained in M-17 broth (Terzaghi and Sandine, 1975) and stored at 4oC until used.

Bifidus-milk manufacture:

Fresh buffaloeís milk was partially skimmed to 3 + 0.1 % fat, heated at 90 ± 1.0 C for 15 minutes, cooled to 45 ± 1 C., inoculated with 10 % active growing culture (Bif. bifidum or Bif. longum), equally distributed into plastic cups (200 ml), covered with aluminum foil using sealing technique and then incubated at 42 ± 1 C till curdling. After complete coagulation, Bifidus-milk samples were kept in refrigerator overnight before analyzed.

Incorporation of GDL in the manufacture of Bifidus-milk:

A portion of fresh buffaloe's milk standardized to 3 + 0.1 % fat, thermized at 90 ± 1.0 C for 15 minutes was used. The milk was pre-cultured for two hours by 5 % Bif. longum or Bif. bifidum before addition of 0.3 % GDL.

Methods:

Chemical analysis:

Total solids, fat content, acidity, pH, total nitrogen (TN) and soluble nitrogen (SN) were measured according to Ling (1963). Lactose content was colorimetrically determined according to Barnett and Abd-El-Tawab (1957). The total volatile fatty acids (TVFA) was estimated by the direct distillation method of Kosikowski (1978). Calcium content was adopted according to the method of Ntailianas and Whiten (1964). Diacetyl was estimated as described by Westerfeld (1945).

Microbiological analysis:

The general plate count technique outlined in the Standard Methods for Examination of Dairy Products (A.P.H.A., 1978) was adopted. Serial dilutions of each sample in citrate buffer were plated on tryptone soya agar (TSA), pH 7.0 + 0.2 (Cook and Brown, 1960) and incubated first at 30°C for 3 days, then at 37°C for another 2 days for count of total bacteria. Bifidobacteria were enumerated according to Dave and Shah (1996) using modified MRS agar medium (m-MRS), supplemented with 0.05 % L-Cysteine HCL and 0.3 % lithium chloride. The plates were incubated at 37oC for 48 hours. For moulds and yeasts, malt extract agar medium (Difco, 1966) and 5 days incubation at 30°C was used.

Rhelogical measurements:

Consistency and viscosity of bifidus milk samples were measured as described by Tamime and Robinson (1983). Syneresis was estimated as the quantity of whey separated from the curd block of Bifidus-milk cup after 60 minutes at room temperature as mentioned by Kammerlehner and Kessler (1980).

Organoleptic scoring:

The organoleptic evaluation of resultant Bifidus-milk was assessed by a panel of 10 persons of staff members of the Dairy Department, Faculty of Agriculture, Al-Azhar University, according to the scheme described by Pearce and Heap (1974).

Nutritional evaluation:

Caloric value:

The caloric value was determined using the calculation method reported by Walstra and Jenness (1984) using the following equation:

$$E = 370\,F + 170\,P + 168\,L + 18$$

Where: E : total energy ; F: fat content (%); P: protein content (%)

L: lactose content (%).

Net utilization calcium (NUCa):

Soluble calcium content was estimated in both milk and resultant Bifidus-milk, then the percentage of net utilization calcium (NUCa) was calculated according to the following equation:

$$NUCa\ (\%) = \frac{\text{Ca in bifidus-milk - Ca in milk x 100}}{\text{Ca in bifidus milk}}$$

RESULTS AND DISCUSSION

The first part of this study was concerned with the use of Bif. longum or Bif. bifidum in the manufacture of Bifidus-milk in order to choice the proper starter.

Table 1 represent the chemical changes in resultant Bifidus-milk during storage at 10 ±1°C for 15 days. It could be gathered from these data that TS, SNF, SN, SN/TN, acidity, diacetyl and TVFA contents were gradually increased in all tested samples throughout storage period. These increases (except acidity) during storage could be attributed to the losses in moisture content of different treatments by evaporation. Whereas, TN, fat, fat/DM, lactose and pH values were slightly decreased. However, the changes in the pH values of resultant product from different treatments coincided with the decrease in lactose content.

Also, it might be gathered that using of Bif. bifidum as starter in making Bifidus-milk improved the quality of the resulted product, where it's samples attained the highest levels of fat, SN, SN/TN, TVFA and diacetyl contents either in the fresh state or throughout the storage period.

The importance of total volatile fatty acids (TVFA) as aroma constituents in fermented milk and the role of diacetyl in the production of a typical flavour of fermented products were previously reported by several investigators (Turcic et al., 1970).

In agreement with literature recordings, the TVFA and diacetyl contents were gradually increased when the storage progressed. It could be also noticed that the highest TVFA and diacetyl contents were detected in Bifidus-milk samples (Treat. II) cultured with Bif. bifidum either when fresh or throughout storage.

Data of microbiological analysis were graphically presented in figures 1-3. From these results, it could be seen that Bifidus-milk made with Bif. longum gave the highest values for the total and bifidobacteria counts, actually log 10.88 and 10.79 respectively, in the fresh state. Moreover, no yeasts or moulds were detected in any of the fresh tested samples for both treatments.

Also, it was evident from the obtained data that the total and bifidobacteria counts in both treatments were gradually decreased with prolongation of storage period. This decline in total bacterial count was likely due to developing of acidity (Badran, 1986 and Mehriz et al., 1993). In addition, it could be also observed that Bifidus-milk made with Bif. bifidum (Treat. II) possessed higher total and bifidobacteria counts at the end of storage, actually log 9.11 and 8.90 respectively, as compared with treatment I.

From Figure 2, it was obvious that yeasts were present in relatively low numbers after 5 days of storage ranged from log 1.43 to log 1.48, however, these values were continuously increased throughout storage and attained the highest population at the end of storage. In the connection, many workers considered that yeasts might be regularded as contaminants and not as constituents of the flora of fermented milk (El-Sadek et al. 1972).

However, the presence of yeast is thought to be due to the sharp developed acidity in resultant Bifidus-milk.

Also, it was of interest to notice that Bifidus-milk made with Bif. bifidum obtained the highest values for bifidobacteria counts throughout storage period, ranged from log 10.28 to 8.90, and in the same time contained the lowest population of yeast varied from log 1.43 to 2.49. These finding may be attributed to the production of antimicrobial agents by bifidobacteria which suppress the growth of yeast (Mohamed, 2002).

The rheological measurements of Bifidus-milk made with either Bif. longum or Bif. bifidum were presented in Fig. (4) & (5) & (6). These results revealed that using Bif. bifidum as starter culture led to produce product with more firm body, more viscous and low ability to retain whey. This finding was true either in fresh state or throughout storage period.

Scoring of resultant Bifidus-milk was generally carried out by means of evaluating flavour, appearance and body & texture. Presented data in Table 2 indicated that the total score points in both fresh Bifidus-milk treatments was similar, actually 10.80 points. Moreover, the obtained results revealed that Bifidus-milk samples after 5 days of storage at 10 ±1oC ranked the highest total score points, being 12.50 points. Furthermore, the average total score points of both tested treatments gradually decreased by prolongation of storage period.

Generally, the organoleptic properties supported the results of chemical analysis. Bifidus-milk cultured with Bif. bifidum possessed the highest values for total volatile fatty acids (TVFA) and diacetyl contents either at fresh state or throughout storage period and in the same time ranked the highest scores for organoleptic assessment.

Evaluation of Bifidus-milk is determined by the nutritive value of milk from which it is made and by the increased digestibility compared with ordinary milk. The data shown in Table 3 declared the main components, caloric value and net utilization calcium of raw milk, Bifidus-milk cultured with Bif. longum and that produced with Bif. bifidum. The caloric value of resultant Bifidus-milk was decreased by 5.82 and 5.60% for treatments I and II respectively due to lactose fermentation to lactic acid. This is advantageous to lactose-intolerance consumers.

In addition, results obtained declared that Bifidus-milk cultured with Bif. bifidum ranked the highest caloric value, actually 272.77 KJ/100g.

Moreover, Bifidus-milk (Treat. II) attained the highest value for net utilization of calcium, being 139.5 % .

Finally, from the previous results, it could be concluded that the manufacture of Bifidus milk from partially skimmed buffaloes milk (3.0+0.1 % fat) with using 10 % Bifidobacterium bifidum, can be recommended as it had well balanced flavor, good organoleptic properties and high nutritive value.

The relatively slow acid production in milk by Bifidobacteria and extending the production time of Bifidus-milk to about 6-7.5 hours became one of the serious problems facing this industry. Many efforts have been carried out to avoid this problem (Salama, 1993; Dave and Shah, 1997 and Murad et al. 1997).

However, a number of authors suggested the production of fermented milk by direct acidification (Bayoumi and Madkor, 1998 and Gamal El-Din et al., 1990). Various acid and acidogens have been used by these workers, but the use of (Glocono Delta Lactone) has a prior use in dairy industry due to gradual release of gluconic acid.

This part of study aimed to investigate the effect of using GDL incorporation with Bif. longum as compared with using Bif. bifidum only on the coagulation time and different properties of resultant Bifidus-milk.

As shown from Table (1), it was interest to notice that the use of GDL in manufacture of Bifidus milk caused a noticeable decrease in the time of production. It has been found that addition of 0.3% GDL incorporation with 5% Bif. bifidum produced Bifidus-milk after 4 hours of incubation at 40oC, whereas neutral Bifidus needs 7 hours for coagulation. Continuously, it was evident that addition of GDL led to reduce the coagulation time by 42.9% as compared with treatments II.

Data of the chemical analysis of Bifidus-milk samples showed an obvious increase in total solids, SNF and acidity while the fat, TN, SN, pH and TVFA contents exerted contrary trend. As a matter of fact, the increase in total solids was one of the principal factors influencing the firmness and body & texture of resulted product.

Extensive investigation concerning microbiological analysis of resultant Bifidus-milk showed that Bifidus-milk produced with Bif. bifidum incorporation with GDL gave the lowest value for the total viable and bifidobacteria counts either in fresh state or throughout storage. This decline in the counts may be due to the increasing the acidity content by hydrolysis of GDL and formation of gluconic acid.

The rheological measurements revealed that addition of GDL increased exudation of whey from the curd (syneresis), produce Bifidus milk with more firm body and more viscous.

Sensory evaluation and the average of score points of Bifidus milk made with or without addition of GDL at fresh stage and throughout storage period are shown in Tables (2). Viewing these results, it might be deduced that Bifidus milk made with bifidobacteria incorporation with GDL gained the lowest grades either when fresh or throughout storage. In this respect, Hempenius and Liska (1964) reported that the use of acidulants including GDL produced acidified dairy products with flat flavor.

As seen in Table (3), addition of GDL caused a slight decrease in caloric values in fresh samples. The same trend of result was also true with respect to NUCa.

In conclusion, from the abovementioned results it could be stated that Glucono-Delta-Lactone (GDL) can be used in a small portion (0.3%) incorporation with Bif. bifidum to reduce the production time to about 4 hours, although slight decrease in organoleptic properties was observed. However, more efforts was found to be necessary to improve the sensoric properties of the resultant Bifidus-milk.

Table (1) Chemical analysis of different treatments of Bifidus milk throughout storage period (average of 3 replicates):

Components	Treatments											
	I				II				III			
	Storage period (days)											
	Fresh	5	10	15	Fresh	5	10	15	Fresh	5	10	15
Total solids %	12.77	12.94	13.12	13.19	12.52	12.76	12.98	13.13	12.61	12.89	13.06	13.21
Fat %	3.40	3.40	3.20	3.00	3.50	3.30	3.20	3.10	3.40	3.20	3.00	2.80
Fat / DM %	28.19	26.27	24.39	23.50	27.95	25.86	24.65	22.85	26.96	24.82	22.97	21.19
Solids not Fat %	9.17	9.54	9.92	10.19	9.02	9.46	9.78	10.13	9.21	9.69	10.06	10.41
Total nitrogen %	0.674	0.670	0.649	0.631	0.654	0.640	0.629	0.612	0.657	0.651	0.620	0.597
TN/DM %	5.28	5.18	4.95	4.78	5.22	5.02	4.85	4.66	5.21	5.05	4.75	4.52
Soluble nitrogen%	0.185	0.190	0.209	0.215	0.175	0.192	0.225	0.240	0.162	0.190	0.210	0.220
SN / TN %	27.45	28.36	32.20	34.07	26.76	30.00	35.77	39.22	24.66	29.19	33.87	36.85
Acidity %	0.85	0.99	1.19	1.24	0.81	0.90	1.09	1.19	0.84	0.93	1.12	1.20
pH	4.75	4.59	4.25	4.15	4.80	4.60	4.33	4.18	4.74	4.57	4.29	4.13
Lactose content %	4.15	3.59	3.37	3.23	4.10	3.60	3.41	3.20	4.01	3.51	3.35	2.91
Diacetyl*	108	122	134	149	111	126	138	152	ND	ND	ND	ND
TVFA**	6.50	9.10	12.20	13.90	6.80	10.00	14.20	15.40	6.60	9.10	11.30	12.30

Treat. I : Bifidus milk made with 10% *Bif. longum*
Treat. II : Bifidus milk made with 10% *Bif. bifidum*
Treat. III : Bifidus milk made with 5% *Bif. bifidum* + 0.3% GDL
ND: Not determined * : mg/100g ** : ml. of NaOH 0.1 N.

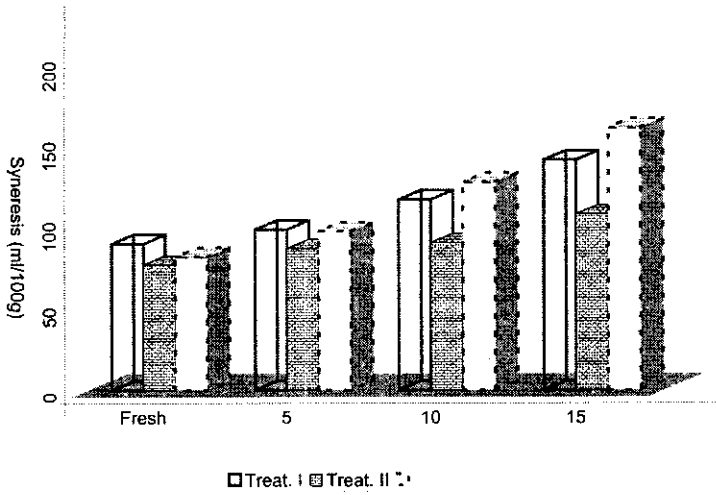

Fig. (1): Total bacterial, Bifidobacteria and Yeast & Mould counts (Log cfu/ml) of Bifidusmilk made with 10% *Bif. longum* throughout storage period Treat. I (average of 3 replicates).

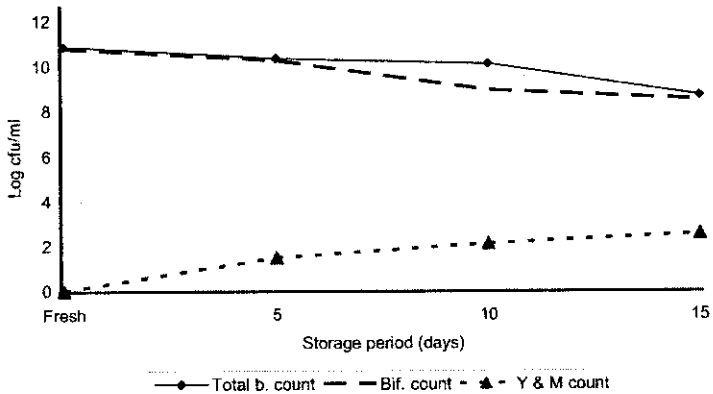

Fig. (2): Total bacterial, Bifidobacteria and Yeast & Mould counts (Log cfu/ml) of Bifidusmilk made with 10% *Bif. bifidum* throughout storage period Treat. II (average of 3 replicates).

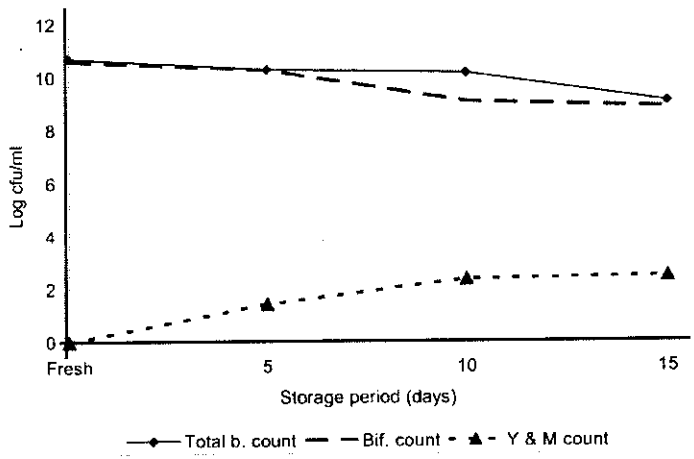

Fig. (3): Total bacterial, Bifidobacteria and Yeast & Mould counts (Log cfu/ml) of Bifidusmilk made with 5% *Bif. bifidum* +3% GDL throughout storage period Treat. III (average of 3 replicates).

Fig. (4): Syneresis of resultant Bifidus-milk from different treatments throughout storage period (average of 3 replicates).

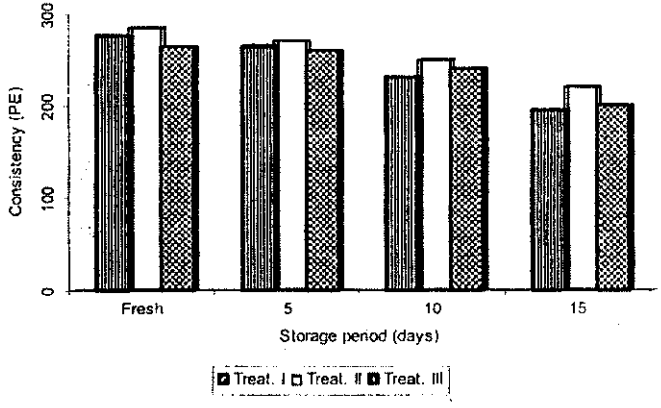

Fig. (5): Consistency of resultant bifidus-milk from different treatments throughout storage period (average of 3 reolicates).

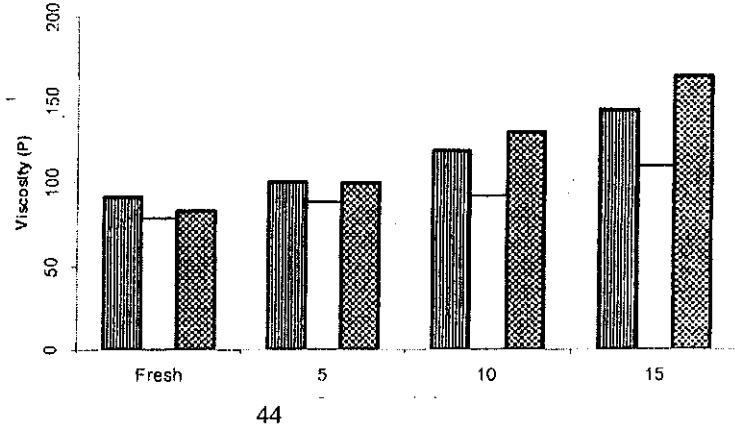

Fig. (6): Viscosity of resultant Bifidus-milk from different treatments throughout storage period (average of 3 replicates).

Table (2) Organoleptic scoring of different treatments of Bifidus milk throughout storage periods (average of 3 replicates):

Properties (Score)	Treatments											
	I				II				III			
	Storage periods (days)											
	0	5	10	15	0	5	10	15	0	5	10	15
Flavor (5)	3.90	4.00	2.70	2.70	4.20	4.50	4.00	3.70	3.40	4.00	2.40	2.70
Appearance (5)	3.50	4.50	3.70	3.40	3.40	4.50	3.40	3.70	3.40	3.75	3.40	3.40
Body&Texture(5)	3.40	4.00	3.30	2.70	3.20	3.50	3.40	3.40	3.60	3.50	3.40	3.70
Total scoring (15)	10.80	12.50	9.70	8.80	10.80	12.50	10.80	10.80	10.40	11.25	9.20	9.80

Treat. I: Bifidus milk made with 10% ***Bif. longum***
Treat. II: Bifidus milk made with 10% ***Bif. bifidum***.
Treat. III : Bifidus milk made with 5% ***Bif. bifidum*** + 3% GDL

Table (3) Nutritional values of different treatments of Bifidus milk (average of 3 replicates):

Components	Milk	Treatments		
		I	II	III
		Fresh	Fresh	Fresh
Total solids %	12.47	12.77	12.52	12.61
Fat %	3.50	3.40	3.50	3.40
Protein %	4.59	4.40	4.27	4.29
Lactose %	4.74	4.15	4.10	4.01
Calcium (mg/100g)	55.40	139	139.9	135.5
Calorie value (kJ/100g)	288.96	272.12	272.77	267.90
NUCa %	-	138.60	139.50	135.10

Milk : partially skimmed buffalos milk (3 % fat)
Treat. I: Bifidus milk made with 10% ***Bif. longum***
Treat. III: Bifidus milk made with 10% ***Bif. bifidum***.
Treat. IV: Bifidus milk made with 5% ***Bif. bifidum*** + 0.3 % GDL
NUCa: Net Utilization Calcium

REFERENCES

American public Health Association (APHA). (1978). Standard methods for the examination of Dairy products. 14th ed., Am-Publ. Health Assoc. Inc., Washington, D.C.; USA.

Badawi, R. M. and EL-Sonbaty A. H. (1997). Viability of Staphylococcus aureus and Escherichia coli in zabady made with Bifidobacteria. Egypt. J. Dairy Sci. 25:217-233.

Badran, S. M. (1986). Study on some starter cultures and its use in soft cheese. M. Sc. Thesis Fac. Of Agric., Cairo, Giza, Egypt.

Barnett, A. J. and Abd El-Tawab, G. (1957). A rapid method for determination of lactose in milk and cheese .J. Sci. food Agric. 8: 437.

Bayoumi, S. and Madkor, S. (1988). The use of Glucono-Delta-Lactone (GDL) in the manufacture of yoghurt. Egyptian J. Dairy Sci., 16: 233- 238.

Cook, A. M. and Brown, M. P. W. (1960) J. pfram. Pharmacol., 12. suppl. 16T118T.

Dave, R. I. and Shah, N. P. (1996). Evaluation of media for selective enumeration of Streptococcus thermophilus, Lactobacillus delbruekii spp. bulgaricus, Lactobacillus acidophilus and bifidobacteria. J. Dairy Sci., 79(9), 1529- 1536.

Dave, R. I. and Shah, N. P. (1997). Effect of cysteine on the viability of yoghurt and probiotic bacteria in yoghurts made with commercial starter cultures. International Dairy J., 7: 537 - 545.

Difco Manual of dehydrated culture media and reagents for Microbiological clinical laboratory.(1986).

El-Sadek, G. M.; Naguib, K. and Negm, A. (1972). Microbiological and chemical studies on zabadi. Milchwissenschaft, 27: 570.

Gamal-El-Din, A.; Shalaby, S.; Noman, A. and Neamat-Allah, A. (1990). A comparative study on using starter and Glucono-Delta-Lactone (GDL) in Zabady making. Com. Sci. & Dev., Vol. 29:11-22.

Hempenius, W. L. and Liska, B. J. (1964). Preliminary Observations on Production of Natural flavor in Chemically Acidified Cream and Skimmilk. J. Dairy Sci., 47: 1099.

Kammerlehner, J. and Kessler, H. G. (1980). Die Molkerei Zeitung welt der Mich, 34: 493. (C. F. Factors affect on stability of physical system of Zabady. El-Naggar 1990. Ph. D. Thesis, Al-Azhar Univ., Egypt.)

Kosikowski, F.A. (1978). Cheese and Fermented Milk Food.2nd Ed.,Published by the author,Cornell Univ., Ithaca,New York., USA.

Ling, E. R. (1963). A text book of Dairy chemistry, vol. 2, practical 3rd Ed.,Chapman and Hall Led., London, U K.

Mehriz, A.M.; Hassan, M. N. A.; Hefny, A. A. and Aziz, A. H. (1993). Manufacture and some properties of ABT cultured and sweet acidophilus milks. Egyptian J. Dairy sci., 21 : 259-272.

Misra, A. K. and Kuila R. K. (1992). Use of Bifidobacterium bifidum in the manufacture of bifidus milk and its antibacterial activity. Lait (Lyon). 72(2): 213-220.

Misra, A. K. and Kuila R. K. (1994). Use of Bifidobacterium bifidum for the manufacture of bio-yoghurt and fruit yoghurt. Indian J. Dairy Sci. 47(3): 192-197.

Mohamed, M. A. A. (2002). Studies on cultured milk beverages. Ph. D. Thesis, Al-Azhar University, Cairo, Egypt.

Murad, H. A.; Fathy F. A. and Abd El-Ghni S. (1997). Growth of Bifidobacterium bifidum in buffalo milk supplemented with peanut milk and some amino acids. Egypt. J. Dairy Sci. 25(1): 75-84.

Ntailianas, H. A. and Whiteny, M. C. I. (1964). Calcein as an indicator for the determination of total calcium and magnesium and calcium alone in the same alquet of milk. J. Dairy Sci. 47: 19.

Pearce, L. E. and Heap, H. A. (1974). Town milk. J. Newzealand Milk Board, 22: 18 (c. f. yoghurt).

Salama, F. M. M. (1993). Chemical and organoleptical properties of biogarde-like product made from lactose-hydrolysed milk. Egypt. J. Dairy Sci., 21 : 273.

Sarkar, S. and Misra, A. K. (1998). Selection of starter cultures for the manufacture of probiotics yoghurt. Egyptian J. Dairy Sci., 26: 295-307.

Tamime, A. Y. and Robinson, R. K. (1983).ì Yoghurt ì Science and Technology 1st ed, pub., Robert Maxwell, M. G. Oxford, New York.

Terzaghi, B. E. and Sandine, W. E. (1975). Improved medium for lactic streptococci and their bacteriophages. App. Microbiol. 29: 807.

Turcic, M. N.; Botic, D. B. and Canic, V. D. (1970). Volatile acids in Yoghurt aroma. Hem. Drust., Beogr., 32: 2.

Walstra, P. and Jennes, R. (1984).Dairy chemistry and physics.John Wiley and Sons Pub. Inc., USA.

Westerfald, W. W. (1945). A calorimetric determination of blood acetoin. J. Biol. Chem. 16 : 495. (C. F. J. Dairy Sci., 77: 2916-2924).