Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 11(1), 247 - 259, 2003

FUNCTIONAL FERMENTED MILK WITH DATE [19]

Nayra Sh. Mehanna[1] and LM. Hosney[1]

ABSTRACT

An attempt was made to produce new types of fermented milk product with yogurt starter and *Bifidobacterium bifidum, Lactobacillus acidophilus* or *lactobacillus reuteri* supplemented with 10 or 20% date. The resultant was assessed for microbiological analysis, pH values and organoleptic properties during 21 days of storage in refrigerator. The bifidus fermented milk made with 20% date had the highest organoleptic scores. In addition, all treatments made with date and any probiotic strain may be described as synbiotic or "functional fermented milk to the presence of assumed therapeutic minimum count of these organisms.

Key words: Functional food, Probiotic, Prebiotic, Date.

INTRODUCTION

Within the definition of functional foods there is a subset of foods believed to be good for health that are produced by or that contain live microorganisms. These foods are called probiotic.

Insert in the role of probiotic for human health goes back at least as far as 1908 when Metchnikoff suggested that man should consume milk fermented with lactobacilli to prolong life **(Hughes and Hoover, 1991)**. Recently, that the interrelationship between intestinal microorganisms and the health benefits deriving from it are beginning to be understood.

Generally it is recognized that an optimum 'balance' in microbial population in digestive tract is associated with good nutrition and health **(Rybka and Kailasapathy, 1995)**. Such microorganisms include lactobacilli and Bifidobacteria. Increasing evidence indicates that consumption of 'probiotic' microorganisms can help in maintaining such a favorable microbial profile and results in several therapeutic benefits. In recent year probiotic bacteria have increasingly been incorporated into foods as dietary adjuncts **(Hattingh and Viljoen, 2001)** for many reason:

1) Control of intestinal infections. 2) Anticarcinogenic activity. 3) Improved lactose digestion in persons as classified as lactose maldigestors, and 4) control of serum cholesterol levels **(Kisla and Unluturk, 1998)**.

One of the most popular dairy products for delivering of viable *Lactobacillus acidophilus* and *Bifidobacterium bifi-*

1- Dairy Science Department, National Research Center, Dokki, Cairo, Egypt.

(Received September 23, 2002)
(Accepted October 28, 2002)

dum cells is bio-yogurt. *Lactobacillus reuteri* is relatively newly recognized species of *Lactobacillus*, which occur in the gastrointestinal tract of humans and animals (**Axelsson *et al* 1989**).

On the other hand, date pectin, dietary fiber and syrup are some of the date substances which can find a plethora of applications as a thickener or gelling agent in processed foods, i.e., confectionery products, jams, table jellies, soft cheeses, yogurt, etc.. Morever, date is a good provider for fiber (about 6.5%), and brown sugar (70%) with negligible fat content. Dietary fiber mainly consists of unmetabolizable polysaccharides, which are excreted taking up malignant tumors. In mature dates, sucrose converts into invert sugar (glucose and fructose). Sugars are in unrefined form and stock up healthy fiber, vitamins and minerals in the fruit. In addition, dates contain seven important vitamins and eleven minerals.

Therefore, the aim of the present study was to produce sympiotic fermented milk contains probiotic bacteria and date as prebiotic according to **Gibson and Roberfroid (1995),** who exchanged "pro" for "pre" which mean "befor" or "for'. They defined prebiotic as "a non- digestable food ingredient that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon".

MATERIAL AND METHODS

Bacterial strains

Streptococcus thermophilus, Lactobacillus delbreuckii subsp.bulgaricus, Lactobacilius acidophilus and *Bifidobacterium bifidum* were obtained from Chr. Hansen's Lab., Denmark. *Lactobacillus reuteri* B-14171 was provided by Northern Regional Research Laboratory, Illinois, USA (NRRL).

Fermented milk manufacture

Samples were prepared by adding 10 or20 percent of blended matured date (w/v) to buffalo's milk (6% fat). A control of buffalo's milk without date was also prepared. Samples were heated at traditional treatment. Each sample was divided into four portions. The first portion was inoculated with 2% yogurt starter only, the second was inoculated with 2% yogurt starter + 5% *Bifidobacterium bifidum* (called as bifidus milk), the third was inoculated with 2% yogurt starter + 2% *Lactobacillus acidophilus* (called as acidophilus milk), where as the forth one was inoculated with 2% yogurt starter + 2% *Lactobacillus reuteri* (called as reutrus milk). The inoculated milk samples were incubated at 42°C for 3 hours followed by storage at refrigerator temperature (5.°C). Three replicates were made from each treatment.

Analytical procedures

Fermented milk samples, from each treatment, were taken when fresh and after 3, 5, 7, 10, 14, and 21 days during storage. Samples were assessed for micrpbiological analyzed, pH values and organoleptic properties.

Microbiological analysis

Streptococcus thermophilus was counted on M_{17} (Oxoid) plates after aerobic incubation at 37°C for 48 hrs.

Lactobacillus delbreuckii subsp. bulgaricus was enumerated according to the method of **Dave and Shah (1996)** using MRS agar (oxoid) adjusted to pH 5.2 and anaerobic incubation at 42° C for 72 hrs.

Lactobacillus delbreuckii subsp. bulgaricus was determined on Lactobacillus selective agar plus 0.2% oxgall (LBSO) **(Gilliland and Walker, 1990).** Plates were incubated at 37°C for 4 days. Enumeration of *Bifidobacterium bifidum* was done according to **Blanchette *et al* (1996)** using modified MRS agar (Oxoid) supplemented with 0.05% L-cysteine-HCl (Merck, Germany). Plates were incubated at 37°C for 48 hrs. In both cases the plates were incubated in an anaerobic environment (BBL Gas Pak, Becton Dickinson, Cockeysville MA, USA).

Lactobacillus reuteri was enumerated on MRS–arabinose agar. MRS basal medium was prepared without dextrose, and 10 ml of membrane-filtered sterile solution of 10%L-arabinose was added per 90 ml of basal medium (1% final concentration) just before pouring the agar medium. Plates were incubated anaerobically at 37°C for 48 hrs.

Molds and yeast were determined on malt extract agar **(Difco, 1966).**

pH values

All fermented milk samples were examined for pH with a pH meter fitted with a punction electrode (digital pH meter).

Organoleptic evaluation

Fermented milk products were judged when fresh and during refrigerated storage by 10 panelists of the experienced staff members of Dairy Science Department, National Research Center. The evaluation includes flavor (50 points), body and texture (30 points) and appearance (20 points).

RESULTS AND DISCUSSION

I - Yogurt

Fig. (1) show that the count of *Str. thermophilus* and *L. delbreuckii subsp. bulgaricus* in yogurt manufactured without date reached the maximum, in fresh one. However the count gradually decreased during storage. The results coincide with those stated by **Sharaf *et al* (1996) and Mehanna *et al* (2002). Hamann & Marth (1984)** reported that the population of viable yogurt starter increased immediately after manufacture of yogurt, then decreased during refrigerated storage of the product.

On the other hand, data given in Fig. (1) show that the increased growth of *Str. thermophilus* and *L. delbreuckii subsp. bulgaricus* during the 3 days of storage followed by a gradual decline up till the end of the storage period. But the count of *Str. thermophilus* and *L. delbreuckii subsp. bulgaricus* in yogurt manufactured with date still more than that in yogurt without date. These results may be due to the effect of nutrational componants of date.

The pH value of yogurt from all treatments gradually decreased during storage period (Fig. 2). The pH of yogurt supplemented with date was higher than those made without date. These results may be due to effect of chemical composition of date.

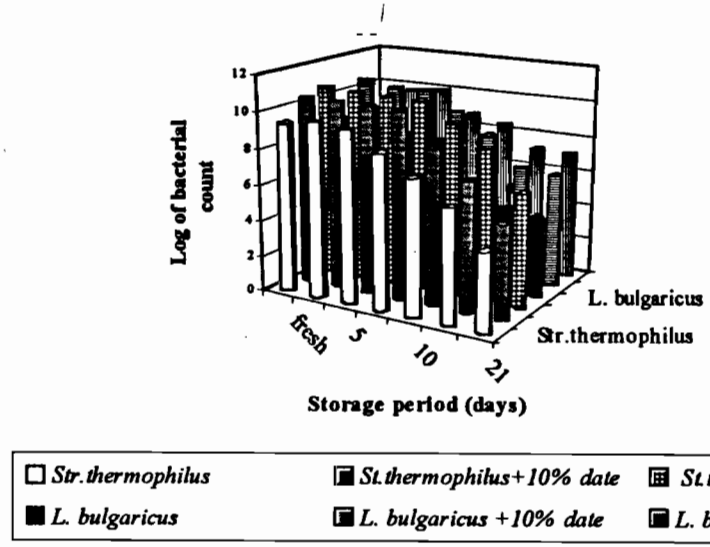

Fig. 1. Change in the viable count of *Str. Thermophilus* and *L. delbreuckii* ssp. *Bulgaricus* dueing storage period of Yogurt supplemented with date

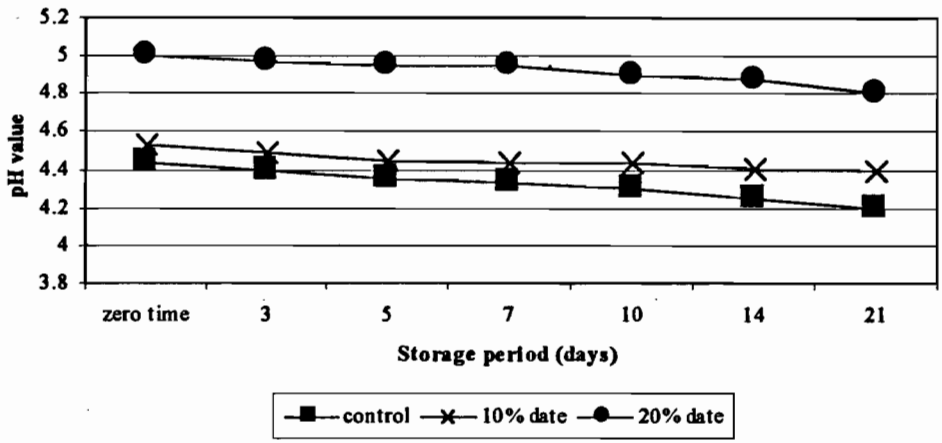

Fig. 2. Changes in the pH values during storage period of yogurt supplemented with date

II- Bifidus milk

As shown in Fig. (3) lactic acid bacteria of all treatments increased gradually during 5 days of storage, and then decreased. This could be due to the fact that during the manufacture process bacterial starter increase in number and continue to multiply for ~ five days, whilst lactose is available in bifidus milk. In addation, it may be noticed that the lactic acid bacteria end the rate of count increasing in bifidus manufacture with 10% date was higher than those manufacture without date, also in bifidus milk 20% date had a pronounced effect on the count of lactic acid bacteria. These results may be due to the effect of some nutrational componants of date and *B. bifidum* on the growth of lactic acid bacteria.

The same trend was observed in growth of *B. bifidum* (Fig. 3). The viability of *B.bifidum* increased in numbers till 3 days of storage. The count slightly decreased after that and sharply decreased after 5 days in all treatments. This result could be due to the presence of lactic and acetic acid, which inhibit the growth of *Bifidobacteria* **(Gomes *et al* 1995).** These results agree with those found by **Sharaf *et al* (1996).**

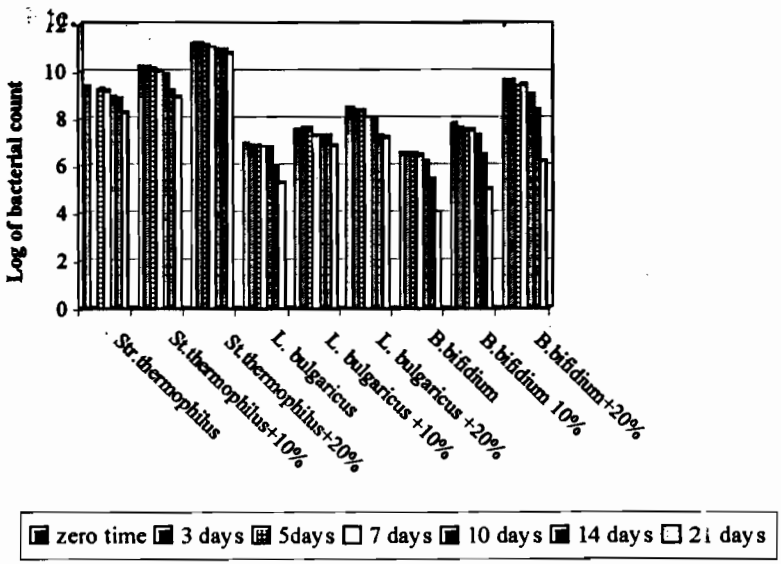

Fig. 3. Changes in the viable count of *Str. Therm* ophilus and *L. delbreuckii* ssp. Bu - garicus and *B. bifidum* during storage during storage period of bifidus milk supplemented with date

On the other hand, the bifidus milk with 20% date had higher count of *B.bifidum,* with 10% date, whereus, the control product (without date) had the lowest number in this respect. These results may be due to the effect of dietary fiber in the date as a bifidogenic factor on the *B. bifidum* as prebiotic **(Gibson and Roberfroid, 1995).** This definition more or less overlaps with the definition of dietary fiber **(Schrezenmeir and De Vrese, 2001).**

Regarding the assumed therapeutic minimum count of *B.bifidum,* 10^7 per gram or milliliter, should be present in a food product in order to meet the requirement of a "probiotic" food as described by the Japans Fermented Milks and Lactic Acid Bacteria Beverages Association **(Ishibashi and Shimamura, 1993).** The resultant bifidus milk with 10 or 20% date showed this count within 14 days of refrigerated storage (Fig. 3). Therefore the bifidus milk with 10 or 20% may be described as "synbiotic" or "functional food" using probiotic and prebiotic.

Data presented in Fig. (4) show that the initial pH value for the bifidus milk without date was the lowest than the other treatment. The pH values of all bifidus tratments gradually decreased along storage. These results agree with those obtained by **Vinderola, *et al* (2000).**

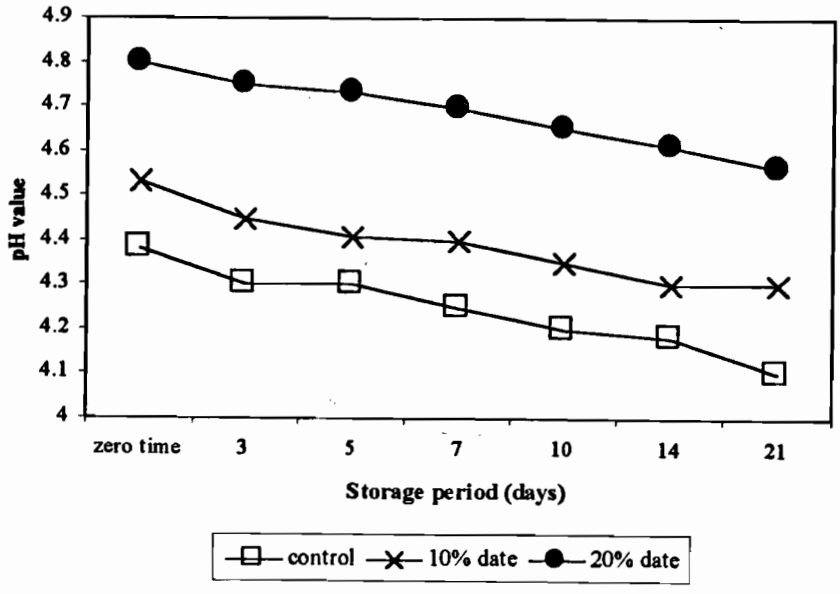

Fig. 4. Changes in the pH values during storage period in bifidus milk supplemented with date

III- Acidopholus milk

Fig. (5) shows the viability of both lactic acid bacteria and probiotic bacteria in acidophilus milk without or with date. Acidophilus produced with date, contained higher lactic acid bacteria than those in acidophilus produced without date. *Str. thermophilus* counts were higher–by at least 1 log order–than those for *L.bulgaricus.* In every case, at end of the storage, the counts of starter bacteria slightly decreased.

Regarding the probiotic microflora, *L.acidophilus* the results obtained showed that its counts decreased during storage. The rate of this loss in cell viability depended on the acidophilus type. Initial count of *L.acidophilus* ranged from 10^7 to 10^8 cfu ml^{-1}, while the final count were 10^6 cfu ml^{-1} (except in acidophilus without date the final count was 10^4). These results may be due to the effect of date as a prebiotic which stimulate the growth of *L.acidophilus.*

As shown in Fig. (6), the pH value of all acidophilus milk slightly decreased. The pH value in the acidophilus milk without date had the lowest value.

IV- Reutrus milk

Change in the viable count of yogurt starter and *L.reuteri* of reutrus milk during storage are present in Fig. (7). Data indicate that the counts of *Str. thermophilusi* and *L. bulgaricus* in all treatment slightly decreased along the storage period.

During storage, slight decline in *L.reuteri* numbers was occurred in all treatments. Whereas, the *L. reuteri* decreased by an average of two logarithmic cycle in reutrus milk without date, while the decrease by an average one logarithmic cycle wasrecorded in other two treatments. Generally, the survival of *L.reuteri* in all treatment tell the end of storage period could be attributed to that this organisms were able to grow at low pH (**Toit *et al* 1998**). In this respect, **Casas *et al*** (1997) reported that *L.reuteri* could survive in various dairy products well beyond 10^5 - 10^6 cfu/ml a 7 to 34 days shelf life.

As shown in Fig. (8), pH value of all reutrus milk gradually decreased throughout storage. This could be due to the formation of more acid during storage.

Moulds and yeasts were also examined in all treatment and were not detected until the end of storage period in reutrus milk. This could be attributed to an inhibtion effect of *L.reuteri* strain against yeasts and moulds (**El-Ziney and Debevere, 1998; Letho and Salminen, 1997 and Effat, 2000**). While, moulds and yeasts were found in other treatmrnts after15 days and increased after21 days.

Organoleptic properties

Data presented in Fig. (9) show that bifidus milk with 20% date had the highest acceptability score followed by bifidus with 10%date, acidophilus with 20%date and reutrus with 20% date. The resultant bifidus milk with date and acidophilus milk with 20% date had a good body & texture, besides it had a pleasant and nuty flavor which were obtained when reutrus milk with 20% date.

Generally, the sensory evaluation results, releved that the resaltant fermented milk products with date, gained the consumer preference for body & texture and flavor.

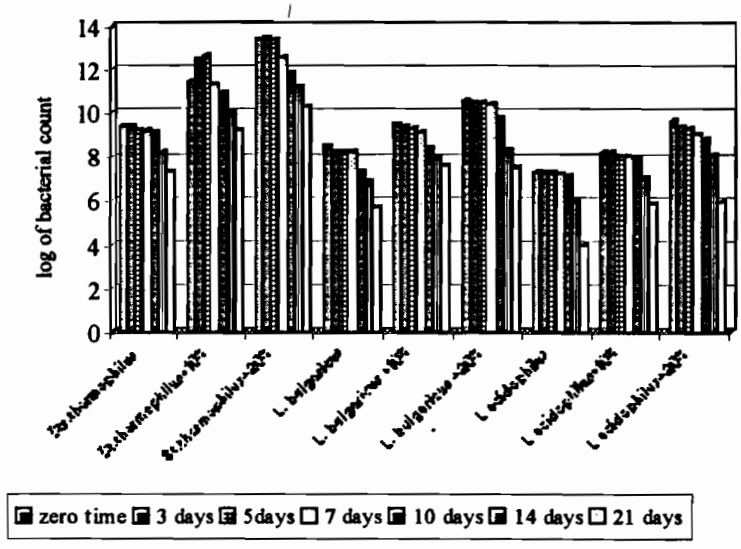

Fig. 5. Change in the viable count of *Str. thermophilus* and *L. delbreuckii* ssp. *Bulgaricus* and *L. acidophilus* during storage during storage period of acidophilus milk supplemented with date

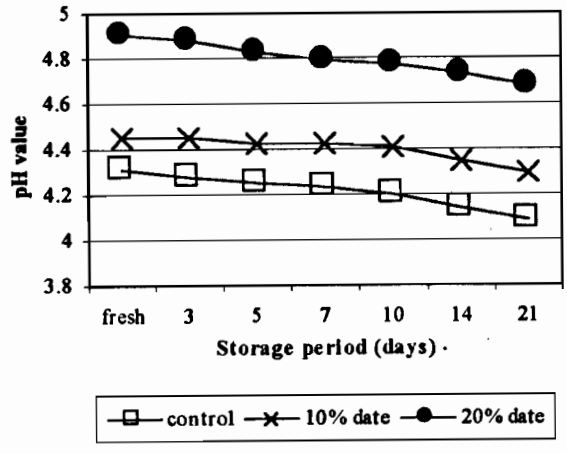

Fig. 6. Changes in pH values during storage period of acidophilus milk supplemented with date

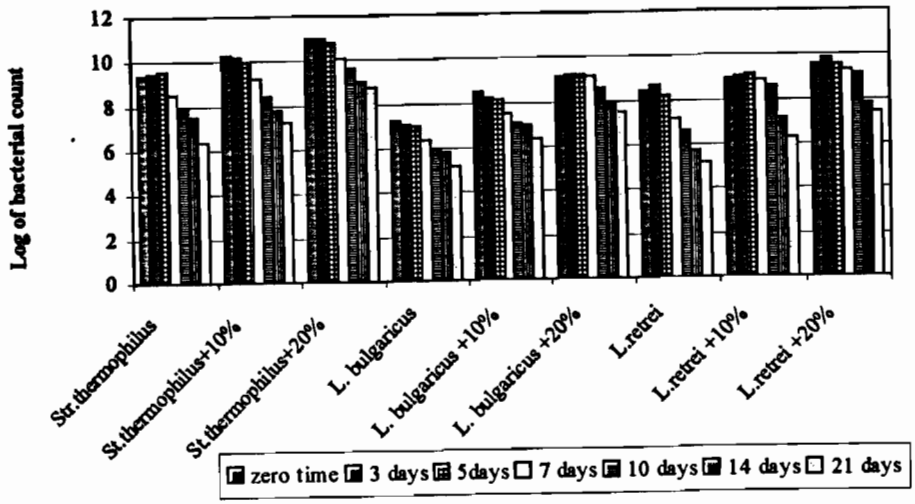

Fig. 7. Changes in the viable count of viable *Str. thermophilus* and *L. delbreuckii* ssp. *Bulgaricus* and *L. reuteri* during storage period of reutrus milk supplemented with date

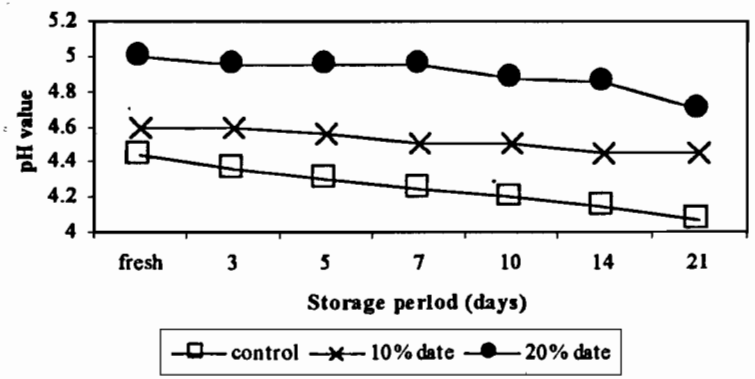

Fig. 8. Changes in the pH values during storage period of reutrus milk supplemented with date

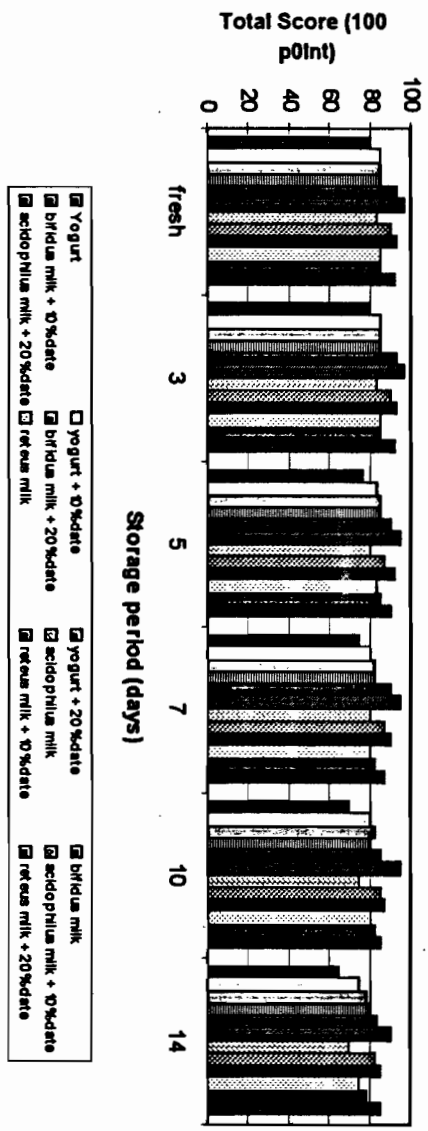

Fig. 9. Organolyptic proprties of new fermented milk supplemented with date

REFERENCES

Axelsson, L.T.; T.C. Chung; W.J. Dobrogosz and S.E. Lindgren (1989). Production of a broad spectrum antimicrobial substance by *Lactobacillus reuteri*. ***Microbial Ecology in Health and Disease 2: 131-136.***

Blanchette, L.; D. Roy; G. Belanger and S.F. Gauthier (1996). Production of Cottage cheese using dressing fermented by Bifidobacteria ***J. Dairy Sci. 79:8-15***

Casas, I.A.; F.W. Edens and W.J. Dobrogosz (1997). *lactobacillus reuteri*; An effective probiotic for poultry, other animals and humans. ***Lactic Acid Bacteria.*** **2nd Ed. pp. 154-156.** Salminen, S. and von A. Wright, eds., Marcel Dekker, Inc., New York.

Dave, R.I. and N.P. Shah (1975). Evaluation of media for selective enumeration of *Streptococcus thermophilus, Lactobacillus delbreuckii* spp. *Bulgaricus, Lactobacillus acidophilus* and *Bifidobacteria. J. Dairy Sci. 79: 1529-1536.*

Difco Manual of Dehydrated Cultures Media and Reagents for Microbiological and Clinical Laboratory Procedures (1966). **9th Ed pp. 65-67.** Difco Lab. Michigan, USA,.

Effat, B.A. (2000). Effect of using *Lactobacillus reuteri* with other probiotic cultures on quality of Domiati cheese. ***Minufiya J. Agric. Res. 25: 445-460.***

El-Ziney, M.G. and J.M. Debevere (1998). The effect of reuterin on *Listeria monocytogenes* and *Escherichia coli* 0157:H7 in milk and cottage cheese. ***J. Food Prot. 61:1275-1280.***

Gibson, G.R. and M.B. Roberfroid (1985). Dietary modulation of the human colonic microbiota. Introducing the concept of prebiotic. ***J. Nutr.125:1401-1412.***

Gilliland, S.E. and K. Walker, (1990). Factors to consider when selecting a culture of *L. acidophilus* as adietary adjunct to produce a hypocholesteralmic effect in humans. ***J. Dairy Sci. 73: 905-911.***

Gomes, A.M.P.; F.X. Malcata; F.A.M. Klaver and H.J. Grande (1995). Incorporation and survival of *bifidobacterium* sp. Strain Bo and *lactobacillus acidophilus* strain Ki in a cheese product. ***Netherlands Milk and Dairy J. 49:71 - 95.***

Hamann, W.T. and E.H. Marth (1984). Survival of *Streptococcus hermophilus* and *Lactobacillus bulgaricus* in commercial and experimental yoghurt. ***J. Food Prot., 47: 781-784.***

Hattingh, A.L. and B.C. Viljoen (2001). Yogurt as probiotic carrier food. ***International Dairy Journal 11:1-17.***

Hughes, D.B. and D.G. Hoover (1991). Bifidobacteria: Their potential for use in American dairy products. ***Food Technology, 45: 74-83.***

Ishibashi, N. and S. Shimamura (1993). Bifidobacteria: research and development in Japan. ***Food Technol. 47:126-135.***

Kisla, D. and A. Unluturk (1998). A new type of fermented milk product manufactured by *lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium longum. Adv. Food Sci. (CMTL) 20: 34-39.*

Letho, E.M. and S. Salminen (1997). Adhesion of two *Lactobacillus* strain, one *Lactococcus* and one *Propionibacterium* strain to cultured intestinal Caco-2 cell line. ***Biosci. Microflora 16:13-17.***

Mehanna Nayra Sh.; B.A. Effat; N.M.A. Dabiza; N.F. Tawfic and O.M. Sharaf (2002). Incorporation and viability of some probiotic bacteria in func-

tional dairy foods II- Hard cheese. *Minufiya J. Agric. Res 27:225-241.*

Rybka, S. and K. Kailasapathy (1995). The survival of culture bacteria in fresh and freeze-dried AB yogurt. *The Australiun J. of Dairy Technology, 50:51-57.*

Schrezenmeir, J. and M. de Vrese (2001). Probiotic, prebiotic and synbiotics- approaching a defination. *Am. J. Clin. Nutr, 73: 3633-3636.*

Sharaf, O.M.; N.Sh. Mehanna; K.E.A. El-Shafei and A.E. Metwally (1996). Effect of using different strains on quality of Labneh. *Annals Agric. Sci., Ain Shams Univ., Cairo, 41:901-912.*

Toit, M. du; C.M.A.P. Franz; L.M.T. Dicks; U. Schillinger; P. Haberer; B. Warlies; F. Ahrens and W.H. Holzapfel (1998). Characterization and selection of probiotic lactobacilli for preliminary minping feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content. *Intern. J. Food Microbiol. 40: 93 -104.*

Vinderola, G.G.; N. Bailo and J.A. Reinheimer (2000). Survival of probiotic microflora in Argentinian yoghurts during refrigerated storage. *Food Research International, 33: 97-102.*

مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية ، جامعة عين شمس ، القاهرة ، ١١(١) ، ٢٤٧ - ٢٥٩ ، ٢٠٠٣

منتجات لبنية متخمرة وظيفية بالبلح

[١٩]

نايرة شاكر مهنا[١] – ابراهيم محمود حسنى[١]

١- قسم الألبان – المركز القومى للبحوث – الدقى – القاهرة

يهدف البحث الى انتاج انواع جديدة من المنتجات اللبنية المتخمرة الوظيفية وذلك باستخدام السلالات التالية

Streptococcus thermophilus, Lactobacillus delbreuckii subsp.bulgaricus, Lactobacillus acidophilus, Lactobacillus reuteri and *Bifidobacterium bifidum*

مع اضافة ١٠% و ٢٠% بلح رطب مفروم وقد تم تحليل المنتجات المتحصل عليها ميكروبيولوجيا وحسيا وتقدير قيم الرقم الهيدروجينى للمنتجات الناتجة وذلك خلال فترة التخزين (٢١ يوما) على درجة حرارة الثلاجة .

وقد أظهرت النتائج حصول المنتج المتخمر المصنع باستخدام سلالة *Bifido-bacterium bifidum* مع ٢٠% بلح. اعلى درجات الجودة الحسية. وقد دلت النتائج على ان جميع المنتجات اللبنية المتحمرة المصنعة بالبلح والسلالات المختلفة ذات قبول لدى المحكمين وقد احتوت على اعداد البكتريا من السلالات المختلفة بالاعداد الموصى بها لأعطاء التأثير الصحى المطلوب الى جانب وجود البلح ذو القيمة الغذائية المرتفعة و تأثيرة الايجابى على نمو السلالات البكتيرية. مما يجعل المنتجات تقع تحت مسمى منتجات لبنية وظيفية أو Synbiotic .

تحكيم: أ.د عصام عثمان فايد
أ.د نبيل محمد مهنا