Annals Agric. Sci., Ain Shams Univ., Cairo, 48(2), 691-702, 2003

VIABILITY OF PROBIOTIC BACTERIA IN FUNCTIONAL FERMENTED MILK CONTAINING HONEY

[48]

Nayra Sh. Mehanna[1]; M.M.E. Salem[1]; Wafaa M. Zaky[1] and Azzat B. Abd El-Khalek[1]

ABSTRACT

The effect of honey on growth and viability of the probiotic bacteria such as *Bifidobacterium bifidum; Lactobacillus acidophilus* and *Enterococcus faecium* was studied to determine whether these organisms were affected by honey compared with control (without honey) during refrigeration. Honey was effective in enhancing the growth rate of two probiotic strains compared with control. Logarithmic phases of growth for two probiotic strains were observed during the first 4 to 8h post inoculation. Growth promotion by honey for all probiotic strains was obtained over the range 0% (control) to 5% concentration of honey as evidenced by decreased doubling time with increased concentration of honey, indicating that *B. bifidum* and *L. acidophilus* grew faster in the presence of honey compared to the controls, while the honey has no effect on *En. faecium*. The resultant fermented milk was assessed for microbiological analysis, acidity and organoleptic properties during 10 days of storage in refrigerator. The new type of fermented milk made with 5% honey had the highest organoleptic scores. Fermented milk made with honey - as a healing agent and probiotic bacteria as *B. bifidum* and *L. acidophilus*, which are considered important to the health of the gastro-intestinal tract (GI) and its therapeutic effect may be described as symbiotic or functional fermented milk.

Key words: Functional fermented milk, Probiotic, Honey.

INTRODUCTION

Honey is a popular sweetener throughout the world. From ancient times, honey was not only used as a natural sweetener but also as a healing agent. Consumers who use honey consider it a healthful product. Many health promoting and curative properties attributed to it are the bases for some traditional folk medicine treatments throughout the world today.

In recent years, with the advent of functional foods, research expanded to include the health promoting aspects of honey. A number of studies on the phytochemicals and antioxidant content of honey and its impact on gastrointestinal health and energy metabolism have identified potential new roles for honey in diets. Honey is a supersaturated sugar solution with approximately 17 percent water. Fructose is the predominant sugar

1- Dairy Science Department, National Research Centre, Dokki, Giza, Egypt.

(Received September 24, 2002)
(Accepted October 22, 2003

at 38.5%, followed by glucose at 31%. Disaccharides, trisaccharides and oligosaccharides are present in much smaller quantities. Besides carbohydrates, honey contains small amounts of protein, (including enzymes), vitamins and minerals. Honey yields 64 calories per tablespoon.

A probiotic may also be a functional food. but more specifically it is a live microbial food supplement that improves the intestinal microbial balance in the host organism. New and novel strains and species have emerged and are likely to be included in our diet. These include *Lactobacillus acidophilus, Bifidobacterium bifidum* and *Enterococcus faecium* (**Salminen and Saxelin, 1996**). The probiotic bacteria used in commercial products today are mainly members of the genera *Lactobacillus* and *Bifidobacterium* (**Heller, 2001**). Probiotics are considered important to the health of the gastrointestinal tract (GI). Clinical studies have associated beneficial effects such as immune enhancement and anticarcinogenicity with presence of probiotic in the GI tract. Honey enhanced the growth of some species of *Bifidobacterium* (**Kajiwara *et al* 2002**), and some species of *Lactobacillus* (**Shamala *et al* 2000**). Thus, recent research has focused on how to ensure its presence in adequate quantities through the addition of probiotics or prebiotics in a suitable carrier such as fermented milk. The challenge when adding probiotic to milk is to maintain a viable, large population during processing and refrigerated storage, not less than 10^7 c.f.u. in order to meet the requirement of a "probiotic" food (**Ishibashi and Shimamura, 1993**).

Therefore, the aim of this study is to produce a functional fermented milk with honey as a healing agent and probiotic bacteria, and the effect of honey on the growth, activity and viability of probiotic bacteria during refrigerated storage of the resulting functional fermented milk.

MATRIAL AND METHODS

Bacterial Strains

Streptococcus thermophilus, Lactobacillus delbreuckii subsp. *bulgaricus, Lactobacillus acidophilus* and *Bifidobacterium bifidum* were obtained from Chr. Hansen's Lab., Denmark. Strain of *Enterococcus faecium* B-2355 was provided by Northern Regional Research Lab., Illinois, USA (NRRL).

Fermented Milk Manufacture

Three batches of fermented milk were prepared by adding 3 or 5% (w/v) of honey (from the local market) to buffalo's milk (from the herd of the Faculty of Agriculture, Cairo University). A control of buffalo's milk without honey was also prepared. Samples were heated to 95°C /20 min. and each sample was divided into three portions. The first portion was inoculated with 2% yoghurt starter + 5% *Bifidobacterium bifidum*, the second portion was inoculated with 2% yoghurt starter + 2% *Lactobacillus acidophilus*, whereas the third one was inoculated with 2% starter yoghurt + 2% *Enterococcus faecium*. The inoculated milk samples were incubated at 42°C for 3 hours. The samples were stored at refrigerator temperature (5°C) two replicates were made from each treatment.

Analytical Procedures

Fermented milk samples were taken periodically when fresh and after 5 and

10 days during storage period. The acidity and acetaldehyde content and organoleptic properties were evaluated.

(a) Microbiological analysis

Streptococcus thermophilus was counted on M17 (Oxoid) plates after aerobic incubation at 37°C for 48 hrs. *Lactobacillus acidophilus* 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 anaerobic environment (BBL Gas Pak. Becton Dickinson, Cockeysville AM, USA).

Enterococcus faecium was enumerated by plating on Kanamycin azide aesculin agar (KAAA, Oxoid) following overnight incubation at 37°C **(Gardiner *et al* 1999)**.

Bacterial growth

Hundred ml of each growth medium were prepared in Erlenmeyer flasks (250 ml volume). Each flask was inoculated with 1 ml of an active culture of the corresponding strain and shaken on a rotary shaker (160 rpm) for 8 hrs at 37°C. Samples (5-ml) were periodically taken from the growing cultures under aseptic conditions to determine the bacterial growth by measuring the O.D. at 620 nm. Using an UV-VIS spectrophotometer (model 8452, Hewlett-Packard).

Growth rate and generation time were calculated from exponential phase using the following equation according to Shin *et al* **(2000)**:

$$\mu = Lnx - Lnxo / t - to$$

$$dt = Ln2 / \mu$$

where: μ = growth rate (hr^{-1})
xo = growth density at zero time
x = growth density after time
dt = doubling time (hr)

(b) Chemical analysis

All fermented milk samples were examined for titratable acidity (T.A %) according to **International Dairy Federation IDF (1991)**. Acetaldehyde content was determined as described by **Lees and Jago (1969)**.

(c) Organoleptic evaluation

Fermented milk products were judged when fresh and during refrigerated storage period by 10 panelists of the experienced staff members of Dairy Science Department, National Research Centre for flavour (50 points), body and texture (30 points), appearance (20 points) of the product as a new type fermented milk with honey (Total score = 100). The experiment was repeated in triplicates and each analysis in duplicate and average results were recorded.

RESULTS AND DISCUSSION

Effect of Honey on Growth Rate of Probiotic Bacteria

Data presented in Fig. (1-a,b,c) indicated that the effect was dependent on the strains type and concentration of honey in the growth medium. Honey was effective

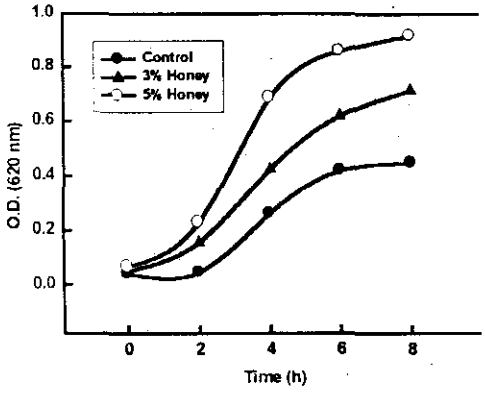

Fig. 1-a: **Effect of honey on the specific growth of *Bifidobacterium bifidum*.**

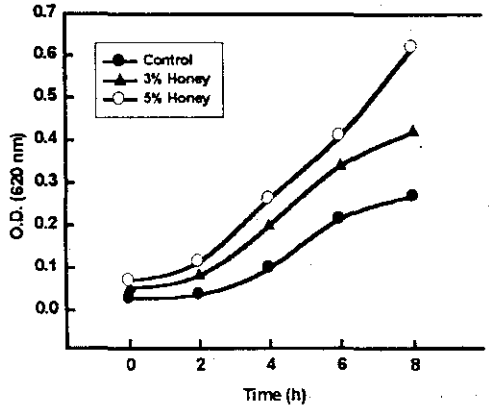

Fig. 1-b: **Effect of honey on the specific growth of *Lactobacillus acidophilus*.**

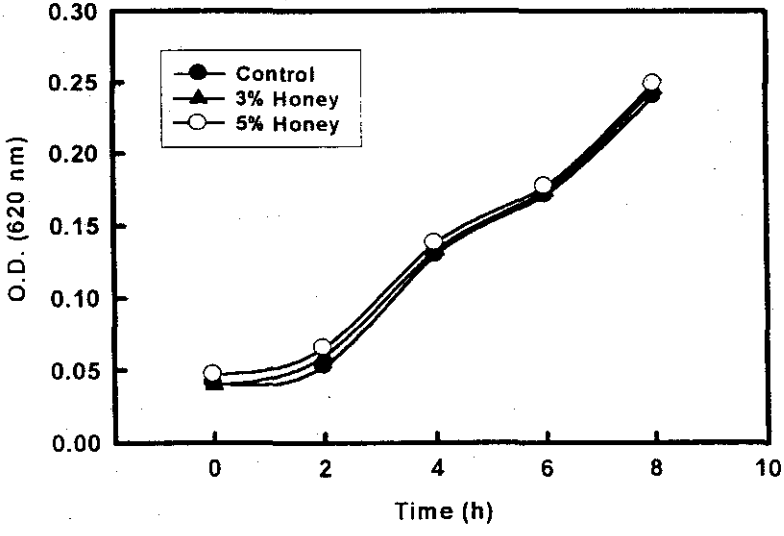

Fig. 1-c: **Effect of honey on the specific growth of *Enterococcus feacium*.**

in enhancing growth rate of probiotic strains as compared with the control. The highest growth was observed by *B. bifidum* achieving cell density of 0.92 O.D., followed by *L. acidophilus* 0.621 O.D. after 8h in case of 5% honey. Logarithmic phase of growth for all probiotic strains were recorded during the first 4 to 8h post inoculation. The doubling time of each probiotic strain in the presence of honey at concentrations 3% and 5% and in the control (without honey) are presented in Table (1). Doubling time was used as a measure of the efficacy of honey in modulating 140 min growth rate. It is clear that growth promotion of probiotic strains by honey was obtained dose dependently over the range 0% to 5% as evidenced by decreased doubling time with increased concentration of honey. The data indicated that the shortest doubling times was 157 min for *B. bifidum* and 140 min for *L. acidophilus* in media containing 5% honey while in the control was 312 and 166 min, respectively. These results are in line with those found by **Dubey and Mistry (1998)** who found that mean generation times ranged from 188 to 322 min for *B. bifidum*. In contrary, there is no effect of honey on doubling time when *En. faecium* was grown in the presence of 5% honey where, it was 245 and 244 in control and with 5% honey, respectively.

These results are consistent with previous reports on the ability of FOS (fructooligosaccharied) - honey contain FOS and other oligosaccharides beside another sweeteners - to stimulate the proliferation of Bifidobacteria relative to other intestinal microflora in vitro culture models simulating the colon **(Gibson and Wang, 1994)**.

Table 1. Effect of honey concentration on doubling time of probiotic bacteria in the selective media.

Species	Doubling Time (min.)		
	0 (Control)	3% Honey	5% Honey
B. bifidum	312	162	157
L. acidophilus	166	152	140
En. faecium	245	243	244

Effect of Honey on Activity and Viability of Lactic Starter and Probiotic Strains in Functional Fermented Milk

1. Production of acetaldehyde

Activity of *B. bifidum* and *L. acidophilus* greatly enhanced when these organisms were grown in the presence of honey as evidenced by acetaldehyde and acidity (as lactic acid) production (Figs. 2 and 3). The effect of acetaldehyde production was more pronounced. The acetaldehyde production was higher in the fermented milk containing *B. bifidum* and *L. acidophilus* as compared with *En. faecium*. These results are in accordance with those of **Salama (2002)**; **Rasic & Kurmann (1978) and Abo-Donia *et al* (1992)**. They reported that adding of *L. acidophilus* or *bifidobacterium* to lactic ferment starter highly activated the production of the acetaldehyde.

However, the results with *En. faecium* did not confirm these results, suggest that the effect of honey on activity of probiotic bacteria may be strain-specific.

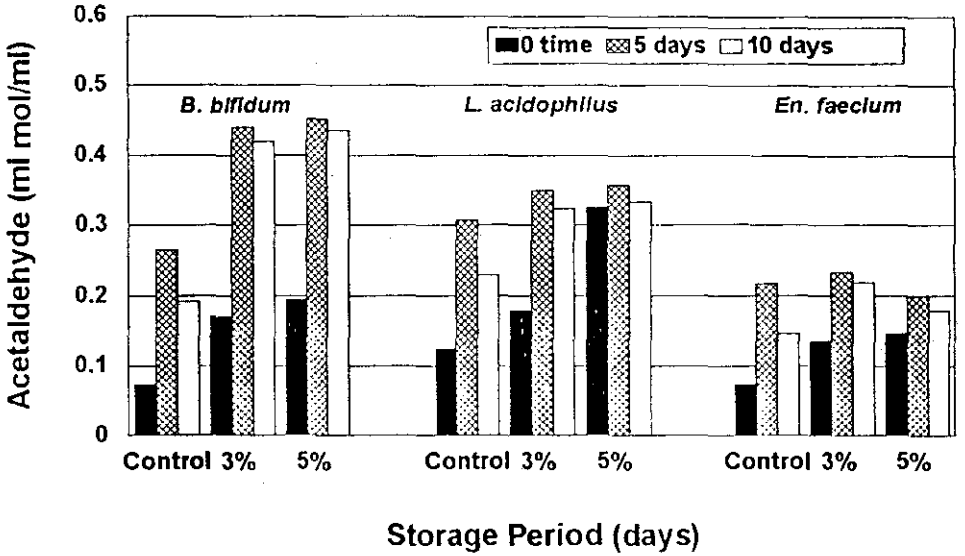

Fig. 2: Effect of honey on the production of acetaldehyde in fermented milk.

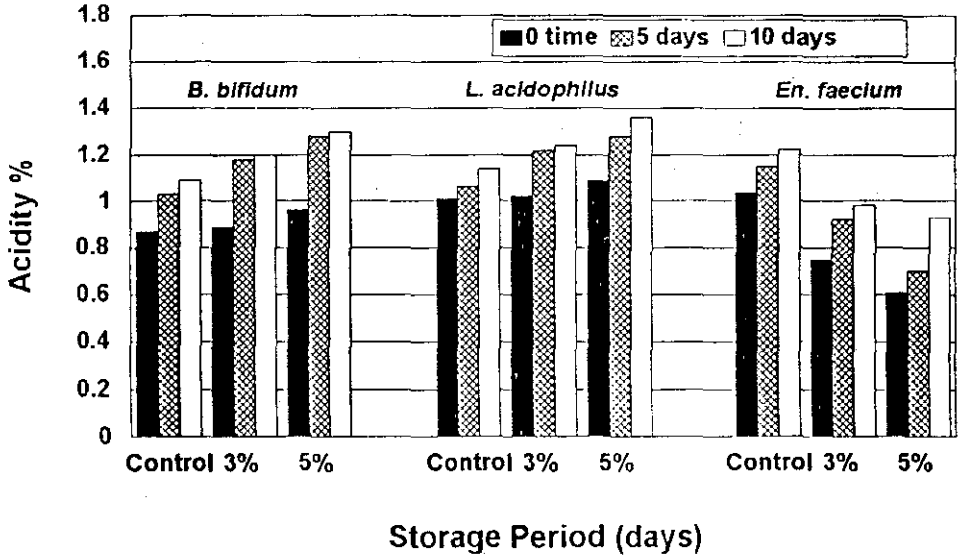

Fig. 3: Effect of honey on the acidity of fermented milk.

2. Production of acidity

Data presented in Fig. (3) show that the acidity values were gradually increased along the storage period in fermented milk made without or with 3 and 5% honey of different types of probiotic strains. While the fermented milk made with *En. faecium* always exhibited the lower values of acidity at any age compared with other strains at the same age. On the other hand, fermented milk made with *B. bifidum* was higher in acidity values as compared with other strains.

3. Viability of probiotic bacteria

The behavior of *Bifidobacterium bifidum* during manufacturing and refrigerated storage period of fermented milk made without or with honey at 3 and 5% honey are shown in Fig. (4). It could be observed that the numbers of *B. bifidum* were increased in all treatments reached maximum in fermented milk made with 5%, 3% and control after 5 days of storage, respectively. This could be due to the fact that during the manufacture process bacterial starter increase in number and continue to multiply for about five days, whilst lactose is available. From data given in Fig. (4) we can noticed that the decrease in population of *B. bifidum* in fermented milk with 3% and 5% honey was lower than control after 10 days of refrigerated storage period. This may be due to the effect of honey as prebiotic or as stimulate the growth of *B. bifidum*.

These results agree with **Chick *et al*** (2001). They reported that the honey has variety of oligosaccharides with low DP (degrees of polymerization) it may be the favored substrate as Bifidobacteria support (bifidogenic factor). Data given in Fig. (5) indicated that the viability of *L. acidophilus* increased in numbers till 5 days of refrigerated storage then sharply decreased after 10 days of storage period in fermented milk without honey, while slightly decrease in numbers was observed in *L. acidophilus* at the end of refrigerated storage period in fermented milk made with 3% or 5% honey. Also, these results could be due to the affect of honey as a prebiotic which stimulate the growth of *L. acidophilus*. However, higher acidity of fermented milk made with honey may be affect on population of *L. acidophilus* (**Gilliand and Rich, 1990**). They found that the maximum population obtained from *L. acidophilus* was significantly higher when the pH during growth was maintained at 5.0 or 5.5 than higher levels of pH.

The viable count of ***En. faecium*** in the fermented milk increased in numbers till 5 days of storage period. The count sharply decreased after that in all treatments. This result indicated that the honey has not higher effect on this strain of *En. faecium* (Fig. 6).

Changes in the viable count of yogurt starter (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) in fermented milk manufactured with honey during storage are present in Figs. (4), (5) and (6). Data indicated that the counts of *L. bulgaricus* and *Str. thermophilus* in all treatments increased up to 5 days and then decreased. The results coincide with those studied by **Sharaf *et al*** (1996) and **Mehanna *et al*** (2002).

These results due to the population of viable yogurt starter increased immediately after manufacture of yoghurt and then decreased during storage refrigeration of the product (**Hamann and Marth, 1984**). On the other hand, data show

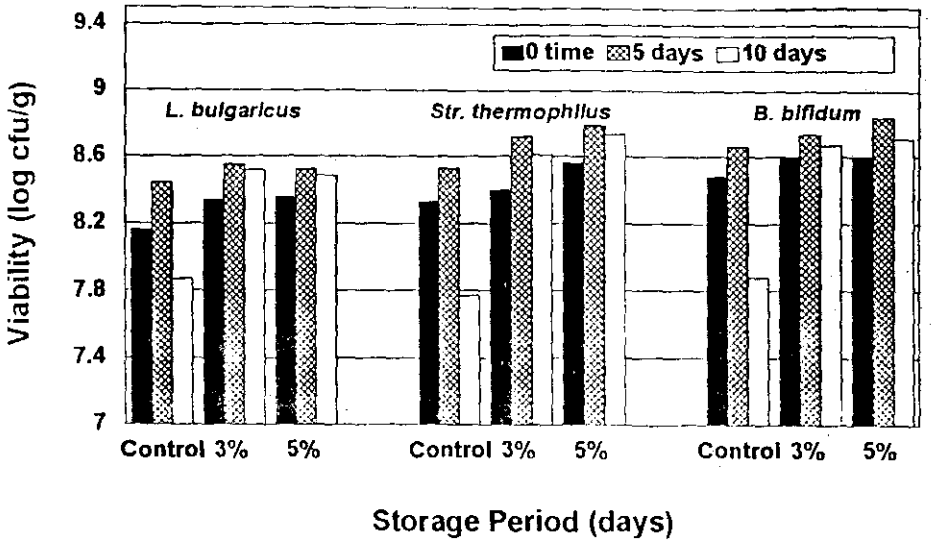

Fig. 4: Viability of different strains in honey fermented milk manufactured with *B. bifidum* during storage period.

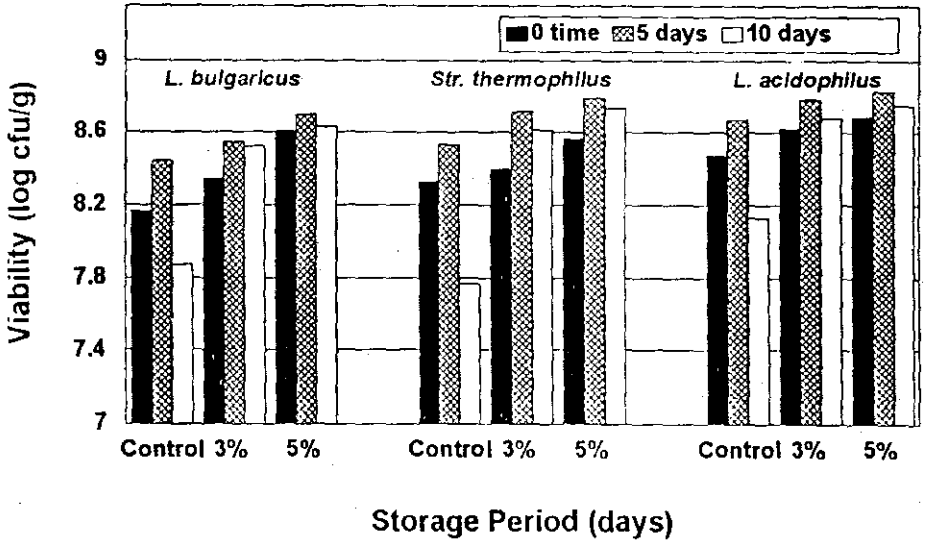

Fig. 5: Viability of different strains in honey fermented milk manufactured with *L. acidophilus* during storage period.

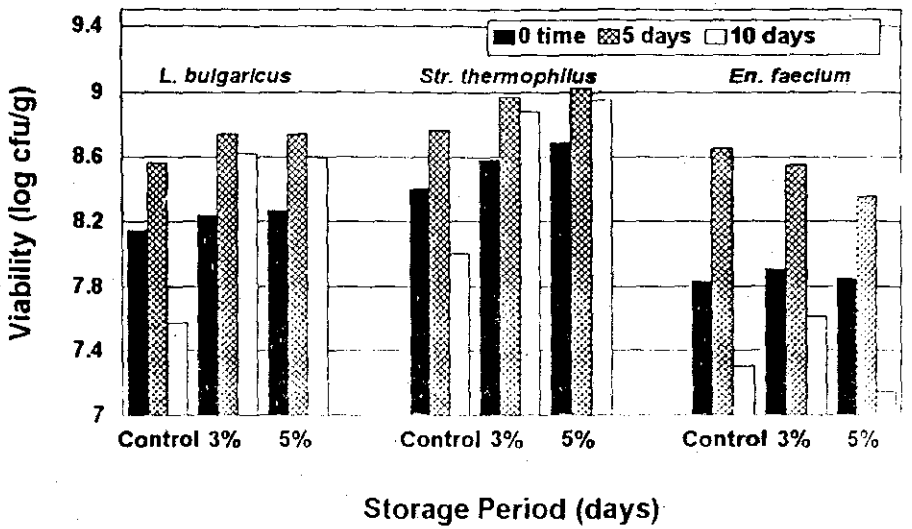

Fig. 6: Viability of different strains in honey fermented milk manufactured with *En. faecium* during storage period.

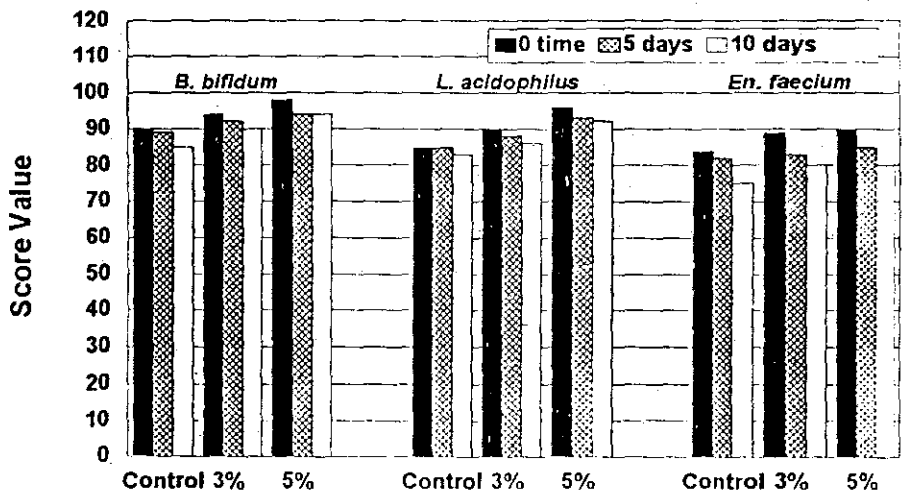

Fig. 7: Organoleptic properties of fermented milk with honey.

that the increased growth of *Str. thermophilus* and *L bulgaricus* during the 5 days of storage followed by gradual decline up till the end of the storage period. But the count of *Str. thermophilus* and *L. bulgaricus* in fermented milk manufactured with honey still more than that in control. These results may be due to the effect of nutritional components of honey especially fructooligosacchariedd (Chick *et al* 2001).

Data present in Fig. (7) show that fermented milk adding *B. bifidum* and 5% honey had highest acceptability scored followed by fermented milk adding *L. acidophilus* and 5% honey.

CONCLUSIONS

From all these results, it could be concluded that the counts of probiotic bacteria and organoleptic scores reached to high score during the storage period at refrigeration. In addition, fermented milk made with honey - as a healing agent and probiotic bacteria and *B. bifidum* and *L. acidophilus*, which are considered important to the health of the gastro-intestinal tract (GI) may be described as symbiotic or function food.

REFERENCES

Abou-Donia, S.A.; I.A. Attia; A.A. Khattab and S.M.C. El-Khadragy (1992). Characteristics of Labneh manufactured using different lactic starter cultures. ***Egypt. J. Food Sci. 1*:1-12.**

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.**

Chick, H.; H.S. Shih and Z. Ustunol (2001). Growth and acid production by lactic acid bacteria and Bifidobacteria grown in skim milk containing honey. ***J. Food Sci. 66(3): 478-481.***

Dubey, U.K. and V.V. Mistry (1998). Effect of bifidogenic factors on growth characteristics of bifidobacteria in infant formula. ***J. Dairy Sci. 79:*1156-1163.**

Gardiner, G.E.; R.P. Ross; J.M. Wallace; F.P. Scanlan; P.J.M. Jagers; G.F. Fitzgerald; J.K. Collins and C. Stanton (1999). Influence of a probiotic adjunct culture of *Enterococcus faecium* on the quality of Cheddar cheese. ***J. Agric. Food Chem. 47:4907-4916.***

Gibson, G.R. and X. Wang (1994). Enhancement of bifidobacteria from human gut contents by oligofructose using continuous culture. ***FEMS Microbial Lett. 118 : 121-128.***

Gilliland, S.E. and C.R. Rich (1990). Stability during frozen and subsequent refrigerated storage by *Lactobacillus acidophilus* grown at different pH. ***J. Dairy Sci. 73: 1187-1192.***

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

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

Heller, K.J. (2001). Probiotic bacteria in fermented foods: product characteristics and starter organisms. ***Am. J. Clin. Nutr. 73 (Suppl): 374S-379S.***

International Dairy Federation IDF (1991). Yoghurt: Determination of titratable acidity potentiometric method ***IDF : pp. 150.***

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

Kajiwara, S.; H. Gandh. and Z. Ustunol (2002). Effect of honey on the growth of acid production by human intestinal *Bifidobacterium* spp.: an in vitro comparison with commercial oligosaccharides and inulin. *J. Food Prot. 65(1): 214-218.*

Lees, G.J. and G.R. Jago (1969). Methods of estimation of acetaldehyde in cultured dairy products. *Aust. J. Dairy Technol. 24: 181-185.*

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 functional dairy foods II. Hard cheese. *Menofiya J. Agric. Res. 27: 225-241.*

Rasic, J.L. and J.A. Kurmann (1978). Yoghurt scientific grounds, technology, manufacture and preparations. *Technology Dairy Publishing House Vanlose, Copenhagen, Denmark, pp. 446.*

Salaw.a, M.M. (2002). Productio. of therapeutic and diabetic stirred yogurt like fermented milk products. *Egypt. J. Dairy Sci. 30: 177-190.*

Salminen, S.J. and M. Saxelin (...). Comparison of successful pr... strains. *Nutrition Today Supplement 32S-34S.*

Shamala, T.R.; Y. Shrijyothi and P. Saibaba (2000). Stimulatory effect of honey on multiplication of lactic acid bacteria under in vitro and in vivo condition. *Letters in Applied Microbiology 30: 453-455.*

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.*

Shin, H.S.; J.H. Lee; J.J. Pestka and Z. Ustunol (2000). Growth and viability of commercial *Bifidiobacterium* spp. in skim milk containing oligosaccharides and inulin. *J. Food Sci. 65(5): 884-886.*

مجلة حوليات العلوم الزراعية، كلية الزراعة ، جامعة عين شمس ، القاهرة ، م(٤٨)، ع (٢)،٦٩١-٧٠٢، ٢٠٠٣

نمو وحيوية بكتريا البروبيوتيك فى الألبان المتخمرة بالعسل كأغذية وظيفية

[٤٨]

نايرة شاكر مهنا[١] - موسى معالى عيد سالم[١] - وفاء محمد زكى[١] - عزات بيومى عبد الخالق[١]

١- قسم الألبان - المركز القومى للبحوث - الدقى - الجيزة - مصر

تم دراسة تأثير العسل الأبيض على نمو وحيوية بعض سلالات البروبيوتيك مثل *Bifidobacterium bifidum* و *Lactobacillus acidophilus* و *Enterococcus faecium* وذلك لإنتاج ألبان متخمرة وظيفية تحتوى على كل من بكتريا البروبيوتيك والعسل لما لكل منهما من تأثير صحى وظيفى للإنسان وخاصة على القناة الهضمية . وقد أدى إضافة العسل إلى البيئة المناسبة إلى لكل سلالة إلى زيادة معدل النمو وكذلك خفض وقت التضاعف لكل من سلالة *B. bifidum* و *L. acidophilus* بينما لم يكن له تأثير على سلالة *En. faecium* وأدى إضافة العسل بنسبة ٥% إلى الألبان المتخمرة المصنعة من بادئ الزبادى بالإضافة إلى بكتريا البروبيوتيك إلى الحصول على منتجات لها صفات حسية جيدة وقد لاقت قبول لدى المحكمين بالإضافة إلى زيادة نمو بكتريا البروبيوتيك مما أدى إلى تواجدها بالأعداد التى تكسبها الصفات الصحية المطلوبة فى الأغذية الوظيفية وبذلك أمكن الحصول على الغذاء الوظيفى المحتوى على كل من العسل وبكتريا البروبيوتيك.

تحكيم: ا.د لطفى فهمى حمزاوى
ا.د أسامة محمود شرف