Global Veterinaria 9 (3): 252-257, 2012
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2012.9.3.6543

Effects of Dill Extract (*Anethumgraveolens L.*) on Growth and Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in Probiotic Milk and Yoghurt

[1]*Mohammad Hossein Marhamatizadeh,* [1]*Farzad Jaafari,*
[2]*Sarah Rezazadeh,* [3]*Elham Ehsandoost and* [1]*Masoud Mohammadi*

[1]Department of Food Hygiene, Veterinary Faculty,
Kazerun Branch, Islamic Azad University, Kazerun, Iran
[2]Department of Food Science, Young Researchers Club,
Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
[3]Department of Food Science, Young Researchers Club,
Kazerun Branch, Islamic Azad University, Kazerun, Iran

Abstract: This study was undertaken to determine the suitability of different doses (0, 0.2, 0.4 and 0.6%) of dill extract (*Anethumgraveolens L.*) for production of probiotic milk and yoghurt by two lactic acid bacteria (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) that can be used in formulation of dairy products. The produced samples were then examined in terms of pH, acidity and microbial count during the incubation period and permanence. In day seven, the products were sensory evaluated. The results of the filled questionnaires in statistical descriptive test were analyzed using SPSS version 17. In the samples containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, it was observed that increased concentrations of dill extract create a favorable taste in yoghurt. The investigation showed that the yoghurt containing 0.6% dill extract had the best for taste, color and insolubility. The control sample and yoghurt containing 0.2% had greater viscosity than the other samples investigated. The bio ability of probiotic bacteria was measured by direct counting method. The shelf lives of products were determined to be 21 days during which the bacterial count decreased but not less than 10^9. There was no significant difference between the samples with *Bifidobacterium bifidum* in terms of sensory features. In this investigation, the effect of probiotic *Lactobacillus acidophilus* and *Bifidobacterium bifidum* milk and yoghurt containing dill extract on cholesterol and triglyceride levels of laboratory rats were evaluated. The results showed that milk with *Bifidobacterium bifidum* was more effective in reducing cholesterol than the milk with *Lactobacillus acidophilus*. However, the probiotic Lactobacillus milk with dill extract was more effective in reducing serum triglyceride in the rats.

Key words: Dill Extract · Probiotic · *Lactobacillus acidophilus* · *Bifidobacterium bifidum* · Triglycerides · Cholesterol

INTRODUCTION

Yoghurt is widely consumed as functional food due to its good taste and nutritional properties (rich in potassium, calcium, protein and vitamin B) and excellent vehicle to deliver probiotics to consumers [1].

In recent years, the probiotic bacteria, as the food additives, have been introduced into numerous foods, of which the dairy products especially yoghurt has played an important role in carrying these bacteria (such as *Bifidobacterium bifidum* and *Lactobacillus acidophilus*). Eating regularly the sufficient amounts of living cells "the minimum treatment" is required if the consumer is to benefit from the probiotic products. The daily recommended amount of the yoghurt containing 10^6 CFU.ml^{-1} probiotic bacteria is 100 gram. It is also very important to investigate the survival of these microorganisms within the interval between storage in refrigerator and consumption [2, 3].

Corresponding Author: Mohammad Hosseinmarhamatizadeh, Department of Food Hygiene, Veterinary Faculty, Kazerun Branch, Islamic Azad University, Kazerun, Iran.

The beneficial effect of probiotics on the human organisms may consist of the improvement of metabolic or physiological processes, as well as medical effects resulting in a reduced risk of incidence of many diseases or their limited duration. Regular consumption of yoghurt is thought to be beneficial in the strengthening of the immune system, improvement in lactose digestion, blood glucose management and the reduction of constipation diarrhea, colon cancer, inflammatory bowel disease and allergies [4, 5].

The beneficial health effects of yoghurt have partly linked to the proteolysis products, produced during fermentation and storage in particular, a group of peptides can lower the blood pressure in hypertensive patients [6]. Phenolic phytochemicals are secondary metabolites of plant origin that constitute an important part of both human and animal diets. Recent studies have shown that phenolic phytochemicals have high antioxidant activity and certain therapeutic properties including anti diabetic and anti-hypertensive activity [7].

In addition, the flavonoid-rich plants have also the ability to inhibit ACE-I activity as shown *in vitro* and *in vivo* studies [8].

In the present study we have studied dill (*Anethumgraveolens*) plant for its phytochemicals that are commonly used in food preparation.

The products from this plant have found their applications as culinary herb or as minor adjuncts to salads (fresh herbs) and herbal teas and as aromatic agents in food, pharmaceutical perfumery and cosmetics industries (essential oils). Significant amount of antioxidants and anti-microbial activity have been demonstrated from both the extracts and essential oils from this plant [9].

Other benefits attributed to consuming this plant include anti-cancer and anti-microbial properties [10-11]. Making of herbal-yoghurts would thus contribute to the development of milk products containing plant phytochemicals. Since yoghurt also exhibits several of these herbs functional properties we have explored the potentials of adding this herb to further enhance yoghurt functional values. Recently, the design and production of plant-based probiotic products have received much attention chiefly due to their natural health benefits (protein, fiber, vitamin and salts) and also because of the variety in their production. Therefore, it seems that the issue of producing probiotic foods with appropriate qualities will be a major research topic for prospective researchers [12].

This study was undertaken to determine the suitability of dill extract (*Anethumgraveolens L.*) for production of probiotic milk and yoghurt by two lactic acid bacteria (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) that can be used in formulation of dairy products. Considering its potential, the purpose of this study was to define the growth and survival of probiotic bacteria in dill milk and yoghurt for possible healthy production.

MATERIALS AND METHODS

Preparation of Probiotic *Bifidobacterium bifidum* Milk Containing Dill Extract at First *Passage*: In order to produce milk containing the probiotic bacterium *Bifidobacterium bifidum*, four containers each containing 1 liter of low-fat sterilized milk (1.5% fat) were considered as our four groups. The starter (*Bifidobacterium bifidum*) was added directly to all the containers, followed by adding dill extract of 0 (Control sample), 0.2, 0.4 and 0.6% to all the containers, respectively and finally they were placed in the incubator at 38°c. The acidity test was performed approximately every 2 hours until reaching 42°Dornic.

The samples were then taken out of incubator and transferred to a refrigerator and stored at 2°C. The produced probiotic milk was evaluated once every 7 days by counting the microbes using direct counting method.

Preparation of Probiotic *Bifidobacterium Bifidum* Yoghurt Containing Dill Extract at the Second Passage: To produce *Bifidobacterium bifidum* yoghurt in this stage, after providing 4 containers, 1 liter of the low - fat sterilized probiotic milk (1.5% fat) from the control group at first passage and the (1.5%) starter of low-fat yoghurt (1.5%) were added to each container.

Different concentrations of dill extract (0, 0.2, 0.4 and 0.6%) were added respectively to the containers and mixed properly so that dill was uniformly dissolved. Afterwards, all the containers were placed in the incubator at 38°C. Approximately every 2 hours, the acidity and pH tests were done until acidity reached 90° Dornic. Then, the samples were taken out of the incubator and transferred to a refrigerator and stored at 2°C. The produced probiotic dill yoghurt was evaluated every 7 days by counting the microbes using direct counting method and after 10 days the yoghurt was evaluated for sensory properties, using questionnaires filled by 50

participants. The respondents were asked to rate the factors of scent, taste and permanence on a scale ranging from very good, good, medium, to weak. The results were analyzed in a statistical descriptive test by SPSS version 17software.

Preparation of Probiotic *Lactobacillus acidophilus* Milk Containing Dill Extract at First Passage: All the same procedures were followed as mentioned above with the difference of using *Lactobacillus acidophilus* instead of *Bifidobacterium bifidum*.

Preparation of Probiotic *Lactobacillus acidophilus* Yoghurt Containing Dill Extract at Second Passage: All the same procedures were followed as mentioned above with the difference of using *Lactobacillus acidophilus* instead of *Bifidobacterium bifidum.*

Having produced the above-mentioned products, we stored 1000 gm of each product in a disposable container placed in a refrigerator for 21 days. During this period, each sample was tested in days 1, 7, 14 and 21 for acidity, pH and sensory properties.

Effect of Probiotic *Lactobacillus Acidophilus* Milk Containing Dill Extract on the Cholesterol and Triglyceride Levels of Rats: In order to evaluate the effect of probiotic *Lactobacillus acidophilus* milk with dill extract on cholesterol and triglyceride levels in laboratory rats, initially the rats that had same condition based on physiological and genetic structure and environmental conditions were prepared. For this purpose, laboratory rats were reproduced in 45 days and then, the adult rats (45 days) cognate, equiponderant (200 gram) and same environmental and nutritional conditions (temperature, moisture, light, bed, water, feed and …) were obtained. Then, rats were divided into 6 groups of 6 pieces:

Group 1: First control group with balance ration.

Group 2: Second control group with high fat ration.

Group 3: Testifier group fed with *Lactobacillus acidophilus* which had no dill extract.

Group 4: Testifier group fed with probiotic *Lactobacillus acidophilus* milk with 0.2% dill extract.

Group 5: Testifier group fed with probiotic *Lactobacillus acidophilus* milk with 0.4% dill extract.

Group 6: Testifier group fed with probiotic *Lactobacillus acidophilus* milk with 0.6% dill extract.

In the beginning of division, blood samples were taken from group 1 as initial control group and sent to the laboratory and the first group was removed. Then the 5 remaining group were fed during 14 days with high-fat ration prepared from pellet and animal fat with the rate of 3 to 1, after 2 weeks, blood samples were taken from the second control group and sent to the laboratory and second group was removed. The other four group received their feeding for 7 days as follows: group 3 was fed with balance ration, group 4 was fed with probiotic *Lactobacillus acidophilus* milk and 0.2% dill extract, group 5 was fed with probiotic *Lactobacillus acidophilus* milk and 0.4% dill extract and finally group 6 was fed with *Lactobacillus acidophilus* milk and 0.6% dill extract.

During this period; milk for every rat was 30 ml daily and after these 7days, blood samples were taken from the 4 remaining groups and sent to the laboratory.

Effect of Probiotic *Bifidobacterium Bifidum* and Dill Extract on Cholesterol and Triglyceride Levels of Rats: All the same procedures were followed as mentioned above with the difference of using *Bifidobacterium bifidum* instead of *Lactobacillus acidophilus*.

Measurement of Serum Cholesterol and Triglyceride Levels in Experimental Rats: To prepare serum samples from the collected blood samples, the samples were centrifuged once or twice with 3500 RPM for five minutes and serum samples were separated. Serum cholesterol and triglycerides measuring kits (Cinnajen, Iran) were used for evaluating serum cholesterol and triglycerides in the experimental rats following the manufacturer procedures.

Statistical Analysis: All the above experiments were repeated three times. SPSS17 was used for one-way analysis of variance for all data and significant differences ($p < 0.05$) among means were determined by the least significant difference test.

RESULTS

Table 1 shows the acidity degrees of dill milk and yoghurt *Lactobacillus acidophilus*, during storage time in the refrigerator and Table 2 shows the growth rates of microbes in dill *Lactobacillus acidophilus* milk and yoghurt at storage time. Table 3 shows the acidity degrees of dill *Lactobacillus acidophilus* milk and

Table 1: The acidity level based on Dornic degree in the dill *Lactobacillus acidophilus* milk and yoghurt within 21-day storage in the refrigerator

Acidity level in Dornic degree									
dill milk	1 day	7 day	14 day	21 Day	dill yoghurt	1 day	7 Day	14 day	21 day
0%	45	45	54	55	0%	91	90	103	94
0.2%	46	48	52	55	0.2%	92	91	104	91
0.4%	48	48	57	57	0.4%	96	93	110	96
0.6%	46	46	53	54	0.6%	94	94	115	91

Table 2: Growth of microbes in the dill *Lactobacillus acidophilus* milk and yoghurt

Dill milk	1 day	15 Day	dill yoghurt	1 day	15 Day
0%	13.5×10^{10}	13.25×10^{10}	0%	7×10^{10}	8.75×10^{10}
0.2%	15×10^{10}	13.75×10^{10}	0.2%	12.5×10^{10}	12.75×10^{10}
0.4%	20.25×10^{10}	14.25×10^{10}	0.4%	6.5×10^{10}	7.75×10^{10}
0.6%	20.5×10^{10}	16.75×10^{10}	0.6%	6.75×10^{10}	10×10^{10}

Table 3: The acidity level based on Dornic degree in the dill *Bifidobacterium bifidum* milk and yoghurt within 21-day storage in the refrigerator

Acidity level in Dornic degree									
Dill milk	1 day	7 day	14 day	21 Day	dill yoghurt	1 day	7 Day	14 day	21 day
0%	42	51	55	59	0%	110	119	121	132
0.2%	44	53	53	58	0.2%	109	127	125	138
0.4%	43	46	48	53	0.4%	92	118	123	129
0.6%	44	48	49	54	0.6%	90	114	122	132

Table 4: Growth of microbes in the dill *Bifidobacterium bifidum* milk and yoghurt

Dill milk	1 day	15 Day	dill yoghurt	1 day	15 Day
0%	65.75×10^{10}	40×10^{10}	0%	14.4×10^{10}	9×10^{10}
0.2%	89.25×10^{10}	45.25×10^{10}	0.2%	21.75×10^{10}	10.5×10^{10}
0.4%	54.75×10^{10}	37.25×10^{10}	0.4%	9.5×10^{10}	6.75×10^{10}
0.6%	75.75×10^{10}	40.75×10^{10}	0.6%	10.75×10^{10}	9.5×10^{10}

yoghurt and Table 4 shows the growth rates of microbes in dill *Bifidobacterium bifidum* milk and yoghurt at storage time in the refrigerator.

DISCUSSION

In the present study, the effects of dill extract on the growth of the bacteria *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in probiotic milk and yoghurt were investigated.

The acidity, pH and survival of the bacteria in dill probiotic milk and yoghurt were evaluated at 2 hours intervals till reaching 42°Dornic acidity degrees for milk and 90°Dornic degree for yoghurt in the incubator at 38°C. At the first hours of production, the *Lactobacillus acidophilus* milk containing 0.6 and 0.4% dill extract reached the acidity of 42°Dornic earliest, followed by 0.2 and 0% milk. Once they reached this acidity level, they were transferred to a refrigerator at 2°c. The storage time in the refrigerator was determined to be 21 days.

In direct microbial counting in first day, the highest counts were sequentially in the samples with 0.6, 0.4, and 0.2% and the controls, indicating the positive correlation between increased bacterial growth and increased dill concentration.

Upon evaluation of the cultured samples on MRS agar media, the same correlation was revealed.

The *Lactobacillus acidophilus* yoghurt with 0.6% dill reached the acidity of 90°Dornic earliest, followed by the samples with 0.4, and 0.2% and the control, Once they reached this acidity level, they were transferred to a refrigerator at 2°c .The storage time in the refrigerator was found to be 21days.

Although the basic feature of the probiotic products consumption is their medicinal effects (bio value), their associated sensory properties are also important. In other words, sensory properties rather than medicinal effects play the most important role in their daily consumptions. Among the probiotic products, fermented ones especially the probiotic yoghurt is popular worldwide for its unique sensory properties [13].

The sensory evaluation was performed by 50 participants for the probiotic *Lactobacillus acidophilus* yoghurt with varying concentrations of dill extract, after seven days. There were significant differences between the samples ($p > 0.05$) and it was shown that the increase of dill extract gives rise to favorable taste, color, scent and thickness.

The minimum required level of probiotic bacteria to be useful for the consumer's body is 10^7CFU.ml^{-1} of living bacteria and the level in the present study was found to be 10^{10}, thus, it could be beneficial for the consumers [14]. Upon evaluation of the samples on MRS Agar, the *Lactobacillus acidophilus* with dill extract had the counts equal to logarithmic 10^8 in day 15 and the sample product with 0.2% dill extract possessed the highest count of bacteria.

Bifidobacterium bifidum milk containing 0.4 and 0.2% dill reached 42°Dornic acidity earliest than others, followed by the milk with 0.6% and finally the control. Once reached 42°Dornic, the samples were transferred to a refrigerator at 2°C. The permanence of the product in the refrigerator was determined to be 21 days during which the acidity of the milk with 0.6% dill extract was lower than other samples.

As revealed in direct microbial counting, the count in day 15 was lower, compared to day 1, for all dill concentrations, but possessed logarithmic coefficient 10^{10}. The bactericidal and inhibitory effect of low pH was stronger for *Bifidobacterium bifidum* than *Lactobacillus acidophilus* and it seems that during the storage time and enhanced fermentations process, decreased pH c aused decreased growth of *Bifidobacterium bifidum*.

At the first hours of production, the *Bifidobacterium bifidum* yoghurt with 0.2 and 0.6% dill extract reached 90°Dornic acidity earliest, followed by the yoghurt sample with 0.4% and the control. They were transferred to a refrigerator at 2°C, once reached the 90°Dornic acidity.

The product permanence in the refrigerator was found to be 21 days. No significant difference was observed in the *Bifidobacterium bifidum* yoghurt with dill extract in terms of color, thickness, taste and scent. The sample with 0.6% was with the highest bacterial counts, as revealed in the evaluation of the samples on MRS Agar medium.

The results also showed that the increased dill concentration in *Lactobacillus acidophilus* milk was positively correlated with reduced serum triglyceride in the rats (0.6%>0.4%>0.2%) and in comparison with the control, the varying concentrations were more effective in reducing serum triglycerides.

The increased dill concentration in probiotic *Bifidobacterium bifidum* milk was negatively correlated with reduced serum triglyceride in the rats (0.6%> 0.4%> 0.2%) and the 0.2% concentration had the greatest effect, even more than the control group.

In sum, the *Bifidobacterium bifidum* milk dill extract was found to be more effective in reducing serum cholesterol in rats than the *Lactobacillus acidophilus* milk. However, the probiotic Lactobacillus milk with dill extract was more effective in reducing serum triglyceride in the rats.

Consumption of probiotic products results in the production of short chain fatty acids which in turn inhibit cholesterol synthesis in the liver and cause the moving of plasma cholesterol to the liver.

The results of the studies addressing the probiotic bacteria have demonstrated the following: The increased concentration of malt and soya caused increase in the microorganism growth and rising acidity level which in turn resulted in shorter incubation time for the desired acidity. In a study on the effects of soya powder on the growth of the bacteria, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, in probiotic products, it was demonstrated that the shelf life for the acidity reaching the desired level during incubation decreased for the milk with both bacteria and combined soya and malt, compared to the milk with only soya. As for the yoghurt with both bacteria, the same results were yielded and incubation time for the yoghurt with malt and soya was decreased [15, 16].

The effect of honey on the growth of the above-mentioned bacteria introduced simultaneously into dairy products and drinks was investigated and the results indicated that yoghurt with only *Lactobacillus acidophilus* tasted sourer than the yoghurt with both bacteria. The products containing *Bifidobacterium bifidum*, compared to those with *Lactobacillus acidophilus*, were with slower growth rate and also tasted less sour and were of longer permanence. They were not of favorable taste when honey concentration increased and the control was of the best taste among all the samples [17].

In another study addressing the effect of cinnamon on the bacterial growth, it was demonstrated that the increased cinnamon concentration promoted the growth of the bacteria in probiotic milk and yoghurt [18].

Taking into account the results of the above-mentioned studies investigating the effects of malt, soya, honey, thyme and mint on the growth of *L. acidophilus* and *B. bifidum*, we can conclude that they all enhance the bacterial growth in dairy products, either when the bacteria are introduced separately or in combination into the products.

The results of the experiments in this work showed that dill extract (*Anethumgraveolens L*) was a suitable support for these intestinal bacteria that were kept viable up to the end of fermentation (21days). All tested *Bifidobacterium bifidum* and *Lactobacillus acidophilus*

were capable of growing well on dill milk and yoghurt without nutrient supplementation. *Lactobacillus acidophilus* had the highest viability in all of the products investigated .The survival of probiotic bacteria in refrigerated conditions for at least 21 days were in number of greater than 10^9cfu. mL^{-1} which is essential if a product should have probiotic properties. It is important to emphasize that all the products possessed excellent stability during 21 days of storage. The sensory scores of the products were high and acceptance. From the foregoing results it can be concluded that dill extract can be successfully used in formulation of dairy products.

REFERENCES

1. Reid, G., M.E. Saunders, H.R. Gaskins, G.R. Gibson, A. Mercenier and R. Rastall, 2003. New scientific paradigms for probiotics and prebiotics. Journal of Clinical Gastroenterology, 37: 105-118.
2. Daranikhosravi, K. and M.K. Kushki, 2008. Probiotics in milk and its products. Marzedanesh publish, 1st ed., pp: 2-3 and 56-57.
3. Marhamatizadeh, M.H., S. Afrasiabi, S. Rezazadeh and Z. Marhamati, 2011. Effect of spearmint on the growth of Lactobacillus acidophilus and Bifidobacterium bifidum in probiotic milk and yoghurt. African Journal of Food Science, 5: 747-753.
4. Yadav, H., S. Jain and S.H. Sinha, 2007. Antidiabetic effect of probiotic dahi con-tainingLactobacillus acidophilus and Lactobacillus casei in high fructose fed rats.Nutrition, 23: 62-68.
5. Adolfsson, O., S. Meydani, N. and R.R. Russel, 2004. Yoghurt and gut function. American Journal of Clinical Nutrition, 80: 245-256.
6. FitzGerald, R.J., B.A. Murray and D.J. Walsh, 2004. Hypotensive peptides from milk proteins. Journal of Nutrition, 134: 980-988.
7. Shetty, K., F. Clydesdale and D. Vattem, 2005. Clonal screening and sprout based bioprocessing of phenolic phytochemicals for functional foods. In K. Shetty G. Paliyath, A. Pometto and R.E. Levin (Eds.), Food biotechnology (pp: 603). New York: CRC Taylor and Francis.
8. Actis-Goretta, L., J.I. Ottaviani, C.L. Keen and C.G. Fraga, 2003. Inhibition of angiotensin converting enzyme (ACE) activity by flavan-3-ols and procyanidins. FEBS Letters, 555, 597e600.
9. Basilico, M.Z. and J.C. Basilico, 1999. Inhibitory effects of some spice essential oils on Aspergillus ochraceus NRRL 3174 growth and ochratoxin a production. Letter of Applied Microbiology, 29: 238-241.
10. Baratta, M.T., H.J.D. Dorman, S.G. Deans, D.M Biondi and G. Ruberto, 1998. Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils. Journal of Essential Oil Research, 10: 618-627.
11. Papadimitriou, C.G., A.V. Mastrojiannaki, A.V. Silva, A.M. Gomes, F.X. Malcata and E. Alichanidis, 2007. Identification of peptides in traditional and probiotic sheep milk yoghurt with angiotensin I-converting enzyme (ACE)-inhibitory activity. Food Chemistry, 105: 647-656.
12. Mortazavian, A.M. and S. Sohrabvandi, 2006. Probiotic and Probiotic foods, Ata publish; pp: 18, 152-155, 202, 210, 213, 219, 235, 371-372.
13. Book-Mortazavian, A.M. and S. Sohrabvandi, 2006. Probiotic and Probiotic foods, Ata publish; pp: 18, 152-155, 202, 210, 213, 219, 235, 371-372.
14. Marhamatizadeh, M.H., R. Rafatjoo, A.R. Farokhi, M. Karmand and S. Rezaazade, 2009. The study of soya extract on the growth of probiotic Lactobacillus acidophilus and Bifidobaceriumbifidum bacteria in probiotic milk and yoghurt. Journal of Veterinary Pathobiology, 1: 23-28.
15. Marhamatizadeh, M.H., M. Karmand, A.R. Farokhi, R. Rafatjoo and S. Rezazade, 2011. The effects of malt extract on the increasing growth of probiotic bacteria Lactobacillus acidophilus and Bifidobacteriumbifidum in probiotic milk and yoghurt. Journal of Food Technology and Nutrition, 8: 78-84.
16. Marhamatizadeh, M.H., I. Rasekh, S. Rezazade and M.R. Kazemi, 2010. Study on honey yoghurt as the carrier of probiotic Bifidibacteriumbifidum. Journal of Veterinary Pathobiology, 1: 31-40.
17. Yaghtin Alireza, 2010. The Study of cinnamon effect on Lactobacillus acidophilus and Bifidobacterium bifidum growth in probiotic milk banana Production. Islamic Azad University, Azad University of Kazerun, pp: 733.