Middle-East Journal of Scientific Research 11 (7): 894-899, 2012
ISSN 1990-9233
© IDOSI Publications, 2012

Effects of Garlic on the Growth of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in Probiotic Milk and Yoghurt

[1]*Mohammad Hossein Marhamatizadeh,* [1]*Masood Mohammadi,*
[2]*Sarah Rezazadeh and* [1]*Farzad Jafari*

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

Abstract: To investigate the effects of varying doses of garlic on the growth of the bacteria *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the probiotic milk (first passage) and yoghurt (second passage), 0.33 gram of the lyophilized probiotic bacteria was separately added to one liter of low fat sterilized milk. The 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*, it was observed that the increased garlic concentration resulted in favorable color, thickness, taste, flavor and scent. However, there were no significant differences between the samples with *Bifidobacterium bifidum* in terms of sensory features. The bio ability of probiotic bacteria was measured by direct counting method. Product shelf life was determined to be 21 days during which the bacterial count decreased but not less than 10^9. It was also shown that the milk with *Bifidobacterium bifidum* was more effective in reducing cholesterol than the milk with Lactobacillus acidophilus. The milk with *Lactobacillus acidophilus* was more effective in reducing triglycerides than the counter part with *Bifidobacterium bifidum*. A positive correlation was observed between the increased concentrations of garlic in the milk with *Lactobacillus acidophilus*.

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

INTRODUCTION

Lactobacillus acidophilus and *Bifidobacterium bifidum* species are well known for their application in fermented milk products. Consumption of these bacteria is frequently related to the health promoting activities of some strains. Strains with beneficial characteristics are referred to as probiotic [1, 2].

Probiotics are beneficial microorganisms that protect the host from diseases and defined as "live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance" [3].

Consumption of dairy products containing Lactobacillus species can aid in the control of serum cholesterol in animal and humans.

Assimilation of cholesterol in the intestines by Lactobacillus species may reduce the amount of dietary cholesterol absorbed in to the body [4].

Because the ability of Lactobacillus species to assimilate cholesterol during growth varies by strain, the Lactobacillus strains for use as a dietary adjunct to potentially reduce serum cholesterol should be carefully selected [4].

Bifidobacteria are a focus of intensive international research for their essential role in fermented food especially for their ability to product various antimicrobial compounds promoting probiotic properties [5].

Recently design and production of probiotic preparations with basic plants are considered for both rule of health nature and variation creation in product and

Corresponding Author: Mohammad Hossein Marhamatizadeh, Departeman of Food hygiene, Faculty of Veterinary, Kazeroun Branch, Islamic Azad University, Kazerun Iran.

consume. It seems that probiotic product of these productions is with relevant quality property taking considerable part of studied searches in the future [6].

The objective of this study was to create a technical method to produce and preserve a kind of fermented probiotic dairy product an with acceptable aroma, while containing reasonable counts of probiotic bacteria.

MATERIALS AND METHODS

Materials: These included garlic plant extract, low-fat yoghurt from supermarket, lyophilized *Lactobacillus acidophilus* & *Bifidobacterium bifidum* (CHR Hansen Company, Denmark) and serum cholesterol & triglyceride measuring kits (Cinnajen, Iran).

Effect of Garlic on the Production of Probiotic *Bifidobacterium bifidum* Milk 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 dried garlic 0% (to the control), 0.1, 0.2 and 0.3% to all other containers, respectively and finally they were incubated at 38°C. The acidity test was performed approximately every 2 hours until reaching 42° Dornic [7]. The samples were then taken out of incubation, transferred to a refrigerator and stored at 2°C [8-10]. The produced probiotic milk was evaluated once every 7 days by counting the microbes using direct counting method [8-10].

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

Different concentrations of garlic (0, 0.1, 0.2 and 0.3%) were added respectively to the containers and mixed properly so that garlic 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 transferred to a refrigerator and stored at 2°C. The produced probiotic galic 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 people. The respondents were asked to rate the factors of scent, taste and thickness on a scale ranging from very good, good, medium, to weak. The results were analyzed in a statistical descriptive test by SPSS version 17 software.

Effect of Garlic on the Production of Probiotic *Lactobacillus acidophilus* Milk at First Passage: The procedure was carried out as described above and instead of *Bifidobacterium bifidum* the bacterium *Lactobacillus acidophilus* was added.

Effect of Garlic on the Production of Probiotic *Lactobacillus acidophilus* Yoghurt at Second Passage: The procedure was carried out as described above and instead of *Bifidobacterium bifidum* the bacterium *Lactobacillus acidophilus* was added.

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

Effect of Probiotic *Lactobacillus acidophilus* Milk Containing Garlic Extract on the Cholesterol and Triglyceride Level of the Rats: In doing so, we initially prepared the rats with the same physiological, genetic and environmental features.

Having bred the rats within a 45 day period, we came to mature rats of the same age (45 days), same sex (female), same weight (200 gram) and same environmental and nutritional features including temperature, humidity, light, living place, water and food. Then, we divided them into six groups of six rats each.

Group 1 was the control group fed with balanced diet. At the beginning, blood samples were collected from group 1 only and transferred to the lab. The remaining five groups (group 2, group 3 and 0.1, 0.2, 0.3 % garlic) were fed with high fat diet prepared from plate and animal fat with 3 to 1 ratio, for 14 days. Afterwards, blood sampling was performed on group 2 and transferred to the lab and the group was excluded.

The other four group received their feeding for 7 days as follows: group 3 received probiotic Lactobacillus acidophilus milk without garlic, group 4 received probiotic Lactobacillus acidophilus milk with 0.1% garlic, group 5 received probiotic Lactobacillus acidophilus milk with 0.2% garlic and finally group 6 received the same milk but with 0.3% garlic.

During this period, 30 ml/day of milk was given to each rat and blood sampling was performed on the four remaining groups and transferred to the lab.

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

Measuring Serum Cholesterol and Serum Triglyceride Levels: To prepare serum samples from the transferred blood samples, the samples were centrifuged once or twice with 3500 RPM for five minutes and serum samples were isolated. Serum cholesterol and triglyceride levels were measured by the used kits according to the manufacturer's instructions.

RESULTS

Table 1 shows the acidity degrees of garlic milk and yoghurt *Lactobacillus acidophilus*, during storage time in the refrigerator and table 2 shows the growth rates of microbes in garlic *Lactobacillus acidophilus* milk and yoghurt at the storage time. Table 3 shows the acidity degrees of garlic *Lactobacillus acidophilus* milk and yoghurt and table 4 shows the growth rates of microbes in garlic *Bifidobacterium bifidum.* Table 5 shows effect of probiotic *Lactobacillus acidophilus* and *Bifidobacterium bifidum* milk with varying doses of garlic on triglyceride and cholesterol level.

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

Acidity level in Dornic degree									
Garlic milk	1 day	7 day	14day	21 day	Garlic yoghurt	1day	7 day	14 day	21 day
0%	47	48	48	53	0%	100	95	114	92
0.1%	47	50	60	58	0.1%	91	91	114	80
0.2%	45	50	72	51	0.2%	90	93	107	85
0.3%	45	54	74	58	0.3%	99	92	108	95

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

Garlic milk	1day	15 Day	Garlic yoghurt	1 day	15 day
0%	19.2×10^{10}	13.8×10^{10}	0%	7.6×10^{10}	4.6×10^{10}
0.1%	12.6×10^{10}	14.2×10^{10}	0.1%	4.2×10^{10}	6.2×10^{10}
0.2%	16.6×10^{10}	19.2×10^{10}	0.2%	8.6×10^{10}	7.2×10^{10}
0.3%	22.2×10^{10}	8×10^{10}	0.3%	6×10^{10}	8.6×10^{10}

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

Acidity level in Dornic degree									
Garlic milk	1 day	7 day	14 day	21 day	Garlic yoghurt	1day	7day	14day	21day
0%	44	46	50	52	0%	85	116	130	94
0.1%	46	52	57	50	0.1%	75	118	130	91
0.2%	43	48	47	60	0.2%	90	117	140	97
0.3%	44	49	48	55	0.3%	90	114	140	98

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

Garlic milk	1day	15 Day	Garlic yoghurt	1day	15 day
0%	37.8×10^{10}	10.8×10^{10}	0%	3.8×10^{10}	3.4×10^{10}
0.1%	54×10^{10}	20×10^{10}	0.1%	11.2×10^{10}	5.4×10^{10}
0.2%	66.8×10^{10}	22×10^{10}	0.2%	5.8×10^{10}	2.8×10^{10}
0.3%	85.6×10^{10}	20.2×10^{10}	0.3%	9.8×10^{10}	3.2×10^{10}

Table 5: Effect of probiotic *Lactobacillus acidophilus* and *Bifidobacterium bifidum* milk with varying doses of garlic on triglyceride and cholesterol level

	Lactobacillus acidophilus		*Bifidobacterium bifidum*	
	Cholesterol	Triglyceride	Cholesterol	Triglyceride
Control group	148	59	148	59
High fat group	224	135	224	135
Non garlic	159	124	159	124
0.1%	141	123	120	133
0.2%	156	76	148	131
0.3%	125	52	68	103

DISCUSSION

In recent years, probiotic bacteria as food additives have been introduced in a good deal of food products, of which milk products and particularly yoghurt plays an important role in carrying such bacteria (e.g. *Bifidobacterium bifidum* and *Lactobacillus acidophilus*). Regular use of an appropriate numbers of living cells is called “minimum treatment”. To enjoy the usefulness of the probiotics, one needs to consume more than 100 gram/day of the yoghurt containing at least 10^6 CFU/ml^{-1} probiotic bacteria. An important issue is sustaining such microorganisms between storage and consumption [11].

Media with milk or whey base could be suitable for the growth and proliferation of such bacteria. Generally, milk is an ideal ground for their growth, because it contains nutrients (i.e. Carbohydrates, fat, proteins, vitamins and minerals), free water and pH close to neutral and unlike other foodstuffs milk does not possess indigestible or hard to digest cells [6].

Garlic, as a medicinal plant, possesses a lot of therapeutic properties of which we could mention antihypertensive, diuretics, anticancer and immunomodulator and lipid reducing agent [12].

In the preset study, the *Lactobacillus acidophilus* milk containing 0.2 and 0.3%, garlic reached the acidity of 42° Dornic earliest, followed by 0.1 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 during which the acidity of the milk with 0.2% garlic was lower than that for other samples.

In direct microbial counting in day one, the highest counts were sequentially in the samples with 0.3, 0.2 & 0.1% garlic in milk and 0.3 & 0.1% in yoghurt compared to the controls, indicating the positive correlation between increased bacterial growth and increased garlic concentration.

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

The *Lactobacillus acidophilus* yoghurt with 0.2% garlic reached the acidity of 90° Dornic earliest, followed by the samples with 0.1 & 0.3% and the control, all of which were then transferred to a refrigerator at 2°C. The storage time in the refrigerator was found to be 21 days during which the acidity level of the yoghurt with *Lactobacillus acidophilus* containing 0.1% garlic was lower than that for other samples.

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 [6].

The sensory evaluation was performed by 50 participants The results of the there were significant differences between the samples ($p > 0.05$) and it was shown that the increase of garlic 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 10^7 CFU.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 [6].

Upon evaluation of the samples on MRS Agar, the *Lactobacillus acidophilus* with garlic extract had the counts equal to logarithmic 10^8 in day 15 and the sample product with 0.3% garlic possessed the highest count of bacteria.

Bifidobacterium bifidum milk containing 0.2 and 0.3% garlic and the control reached 42° Dornic acidity earlier than milk with 0.1%. 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 0.1% garlic was lower than that for the counterparts.

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

The *Bifidobacterium bifidum* yoghurt with 0.1 and 0% (Control) garlic reached the 90° Dornic acidity earlier than the yoghurt samples with 0.2 and 0.3%. The product permanence in the refrigerator was found to be 21 days during which the acidity of the yoghurt with 0.1% garlic was lower than that for other samples.

No significant differences were observed in the *Bifidobacterium bifidum* yoghurt with the various garlic extracts in terms of color, thickness, taste and scent. The sample with 0.1% and the control were with highest bacterial counts, as revealed in the evaluation of the samples on MRS Agar medium.

The increase of garlic concentration in *Lactobacillus acidophilus* milk was positively correlated with reduced serum cholesterol of the rats. However, the two concentrations 0.1 and 0.3% were more effective in reducing serum cholesterol, compared to the controls.

The increased garlic concentration in *Lactobacillus acidophilus* milk was positively correlated with reduced serum triglyceride in the rats (0.1>0.2> 0.3%) and in comparison with the control.

The increased garlic concentration with 0.1 and 0.3% in *Bifidobacterium bifidum* milk was positively correlated with reduced serum cholesterol (0.2> 0.1>0.3%). The increased garlic concentration in probiotic *Bifidobacterium bifidum* milk was positively correlated with reduced serum triglyceride in the rats (0.1> 0.2> 0.3%) and the 0.3% concentration had the greatest effect.

In sum, the *Bifidobacterium bifidum* milk with garlic extract was found to be more effective in reducing serum cholesterol in rats than the *Lactobacillus acidophilus* milk. However, the probiotic Lactobacillus milk with garlic 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 inhibit cholesterol synthesis in the liver and cause the moving of plasma cholesterol to the liver [6].

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 [8, 9].

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 the yoghurt with only *Lactobacillus acidophilus* tasted more sour 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 [10].

In another study addressing the effect of spearmint on the bacterial growth, it was demonstrated that the increased spearmint concentration promoted the growth of the bacteria in probiotic milk and yoghurt [13].

In another study addressing the effect of juice on the bacterial growth, it was demonstrated that the increased juice product promoted the growth of the bacteria in probiotic orange and apple [14].

As a final conclusion we can say, the samples were more lasting using *Lactobacillus acidophilus* bacteria than *Bifidobacterium bifidum.*

ACKNOWLEDGEMENT

Our special thanks go to Hassan Khajehei for his copy editing of the manuscript.

REFERENCES

1. El-Banna, H.A., H.Y. El-zorba, T.A Attia and A. Abd Elatif, 2010. Effect of probiotic, prebiotic and symbiotic on broiler performance. World Applied Science Journal, 11: 388-393..
2. Tabatabaie, F. and A. Mortazavi, 2008. Influence of Lactulose on the survival of probiotic strains in yoghurt., World Applied Scince Journal, 3(1): 88-90.
3. Saad, Amal S., Habashy, M. Madlen, Sharshar and M. Khadiga, 2009. Growth response of the freshwater prawn, macrobrachium rosenbergii (De Man), to diets having different levels of biogen., World Applied Scince Journal, 6(4): 550-556.
4. Raghavan, C.M., A. Nanda, R. Yuvaraj, D.J. Mukesh Kumar, Senthil Murugan and R. Balji Raja, 2011. Assimilation of cholesterol by Lactobacillus species as probiotic. IDOSI Publication, World Applied Scince Journal, 14(4): 552-560.
5. Zinedine, A. and M. Faid, 2007. Isolation and characterization of strains of Bifidobacteria with probiotic proprieties In vitro., World Journal of Dairy & Food Scince, 2(1): 28-34.
6. Mortazavian, A.M. and S. Sohrabvandi, 2006. Probiotic and Probiotic foods. Ata publish; 18, 152-155, 202, 210, 213, 219, 235, 371-372.
7. Standard institute and industrial search of Iran. 2006. Milk and dairy: Finding acidity and pH-Test method, Notional industrial number, 9985.
8. 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 Bifidobacterium bifidum in probiotic milk and yoghurt. Journal of Food Technology & Nutrition, 8: 78-84.
9. 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 Bifidobacerium bifidum bacteria in probiotic milk and yoghurt. Journal of Veterinary Pathobiology, 1: 23-28.
10. Marhamatizadeh, M.H., I. Rasekh, S. Rezazade and M.R. Kazemi, 2010. Study on honey yoghurt as the carrier of probiotic Bifidibacterium bifidum. Journal of Veterinary Pathobiology, 1: 31-40.
11. Darani Khosravi, K. and M.K. Kushki., 2008. Probiotics in milk and its products. Marze danesh publish, 1st ed.,: 2-3 & 56-57.

12. Jahan Ara, F. and S.H. Haerizadeh, 2002. Information and usage drugs herbaceous official Iran. 1st ed, 581-583.
13. 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 yogurt. African Journal of Food Science, 5(13): 747-753.
14. Marhamatizadeh, M.H., S. Rezazadeh, F. Kazemeini and M.R. Kazemi, 2012. The study of probiotic juice product conditions supplemented by culture of Lactobacillus acidophilus and Bifidobacterium bifidum., Middle-East Journal of Scientific Research, 11(3): 278-295.