J. Appl. Environ. Biol. Sci., 4(11S)191-197, 2015

ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Effect of Vanillin on *Lactobacillus acidophilus* And *Bifidobacterium bifidum* And Evaluation of Its Physicochemical and Sensory Properties in Probiotics Yoghurt

Zamzam Rezazadeh[1*]; Mohammad Hossein Marhamatizadeh[2]; Mohsen Radi[3]

[1, 3] Department of Food Science and Technology, Yasuoj Branch, Islamic Azad University, Yasouj, Iran.
[2]Food Hygiene Department, Veterinary Faculty, Kazerun Branch, Islamic Azad University, kazerun, Iran.

Received: October 29, 2014
Accepted: December 31, 2014

ABSTRACT

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 *B. bifidum* and *L. acidophilus*). The purpose of this study, determines the effects of different doses of Vanillin at 0.2%, 0.4% , 0.6% and 0.8%(w/v), in two passages After and Before incubation, on the growth of two probiotic bacteria (*Bifidobacterium bifidum* and *Lactobacillus acidophilus*) in produced yoghurt. The vanillin yoghurt was produced by Tamime, standard methods. The products were then examined in terms of sensory method, pH, acidity and microbial counting during the incubator setting period and their respective permanence. The results in statistical–descriptive test were analyzed by using SPSS software version 16 system. It was repeated for three times respectively. All tested strains showed a good growth rate in vanillin yoghurt without added nutrients. There were significant differences between the control yoghurt on the one side and the yoghurts with 0.2%, 0.4 and 0.6% vanillin concentration with the same bacterium on the other side in terms of sensory properties and shelf life ($P<0.05$). The After yoghurts were significantly different in taste, thickness and flavor with before yoghurts ($P<0.05$), but no significant difference was detected between them.

KEYWORDS: Probiotic; *Bifidobacterium bifidum*; *Lactobacillus acidophilus*; Vanillin.

INTRODUCTION

A functional food used by consumers is the one with additional basic nutritional properties, at least an established distinct healthful property and recommended are among functional foods. One of the significant points in relation to the selection and production of functional purpose fully by producers or foods scientists. Milk and its products, particularly the respective fermentable products foods is their immune and safe consumption. Nowadays, because of confirmed undesirable results of imbalanced and bad eating in human societies, tendency toward the production and consumption of different functional foods have considerably increased. Probiotics, as a kind of new and favorite functional products are great importance. The distinctive feature of such products is their inclusion of some microorganisms, that is, bacteria rather than synthetic compounds in them [23].

Lactobacillus and *Bifidobacterium* are normal human intestinal flora, probiotics [27] are the production of short-chain fatty acids reduction of colon cancer, improve the intestinal microbial balance, resulting in the inhibition of bacterial pathogens, improving the immune system and lowering serum cholesterol levels [25].

Probiotics are recognized for their applications in dairy products, particularly yoghurts and the market for these products is still rising. To achieve the health benefits of probiotics should be their number to 10^6 log CFU/g. This is a standard for the production and sale of products whose names they are given probiotics [18].

However, in commercial products various probiotic lactobacilli and bifidobacteria show a decline in their viability during product's shelf life [24, 12].

Recently biotechnology has led to a great improvement in the food industry. And food diversity is increasing. Among these, probiotic yogurt, are known as a suitable environment for probiotics growth. Also, according to the therapeutic effect, this product has been popular among people [10].

Lactic acid bacteria and its metabolites improve the quality and increase the shelf life of fermented food products [30].

***Corresponding Author;** Zamzam Rezazadeh, Department of Food Science and Technology, Yasuoj Branch, Islamic Azad University, Yasouj, Iran. E.mail: Zam.1366_11_4@yahoo.com

Since LAB are able to convert lactose and other carbohydrates into lactic acid is used in the food industry [11]. LAB because of their important role in fermentation foods is the attention of many in international research. Its ability to produce various anti-microbial compounds promoting probiotic properties [28]. That includes antitumoral activity [6], reduction of serum cholesterol [5], alleviation of lactose intolerance [6], stimulation of the immune system [9], and stabilization of gut micro flora [4]. That is employed in the manufacturing of fermented milk to improve its texture and viscosity [24]. Factors such as: the used strains, storage time and temperature, culture conditions, antagonism among cultures present, initial counts, hydrogen peroxide and oxygen contents in the medium and the amount of organic acids in the product responsible for the viability of probiotic organisms [15].

Probiotic organisms especially bifidobacteria grow slowly in milk due, in part, to their lack of proteolytic activity, thus requiring the incorporation of essential growth factors such as peptides and amino acids to enhance their growth [1].

Considerable studies have been conducted to growth and survival improved of probiotics in yogurt fermentation was conducted as vitamin enriched protein hydrolysate, amino nitrogen and whey protein concentrate [2, 14] to enhance the flavor as well as the nutritional quality [26].

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 [16]. Therefore, it seems that the issue of producing probiotic foods with appropriate qualities will be a major research topic for prospective researchers.

Sensory properties rather than medicinal effects play the most important role in their daily consumptions. Among the fermented probiotic products, the probiotic yogurt is popular worldwide for its unique sensory properties [23, 24].

Iran possesses a very rich source of such plants and herbs in the world in terms of variety and amount. The essence plants play a critical role in human life, and have been used for long by Iranians.Vanillin is obtained from a tropical orchid and widely used in foods as a flavoring substance, Vanillin, a phenolic compound that has broad anti microbial properties [17,8]. Scientists in the1994 observed the antibacterial vanillin effect on other systems, this material decreases survival of yeast and fungal hyphae during the storage of various products [3, 19].

The purpose of this study is to evaluate the effect of Different percentages of Vanillin (0, 0.4, 0.8 and 1.2 %) on the growth and viability of Bifidobacterium bifidum and Lactobacillus *acidophilus* in probiotic milk and yoghurt during refrigerated storage.

MATERIALS AND METODS

Materials

Materials included vanillin, Low-fat sterilized milk (1.5% fat content).

Probiotic lyophilized packages of Lactobacillus acidophilus nu-trish® LA-5 and Bifidobacterium bifidum nu-trish® BB-12 were prepared from (CHR Hansen Company, Denmark) and MRS Agar culture medium was used for carrying out the microbial test (Merk Company, Germany).

Sample Preparation

In order to produce the probiotic milk containing probiotic bacterium *B. bifidum*, *L. acidophilus,* and to mix them, four containers, containing 1 L of low-fat milk were Labeling as four groups. 1.7×10^{12} CFU.ml^{-1} starter was added directly to all the containers, for *L. acidophilus* milk, 4.9×10^{12} CFU.ml^{-1} for *B. bifidum* milk and 3.3×10^{12} CFU.ml^{-1} for mixing them. This was incubated at 38 ° C and acidity measurements were performed at different times until reaching 42°Dornic (Standard and industrial search of Iran). Then the samples were taken out of incubator and carried to a refrigerator and stored at 2°C. This process was done for each one of the starters separately.

Preparation of Before Passage

The second passage, in order to produce probiotic yoghurt, 4 containers were provided and 1 liter of low-fat milk and 15 ml of probiotic milk from the first passage and different percentage of vanillin (0, 0.4, 0.6 and 0.8%) were added respectively to the containers and mixed properly, all samples were incubated at 38 ° C and acidity measurements were performed at different times until reaching 72°Dornic. Finally, the samples were placed in a refrigerator at 2 ° C.

Preparation of After Passage

The purpose of After Incubation Sample, Add to vanillin has Stirred probiotic yogurt. In this passage different percentage of Vanillin (0, 0.2, 0.4 and 0.6%) were added respectively to the containers containing stirred probiotic yoghurt, from the second passage control samples.

Having produced the above-mentioned products, all products were stored in a refrigerator for 28 days. During this period, each sample was tested for acidity, pH, and sensory properties and microbial counts after 1, 7, 14, 21 and 28 days.

In this study, bacterial counts were performed by the direct method and cultured on MRS Agar. Furthermore after 10 days samples were evaluated for sensory properties, (aroma, scent, color, thickness and taste) the questionnaire by 30 peoples. The respondents were asked to rate the factors on a scale ranging from very good, good, medium, weak and very weak. The results were analyzed in a statistical descriptive test by SPSS version 16 software.

Statistical Analysis

All the above products and experiments were repeated three times. And SPSS software was used to analyze data.

RESULTS AND DISCUSSION

This is indicative of fast growth probiotics during the initial stages of incubation because the yoghurt starter experiences fast growth rate exceeding that of *L. acidophilus*. The otherwise condition happens if *L. acidophilus* is adapted to the condition during the first passage and has sufficient growth [3].

Adding traditional yogurt starter can be created competition between traditional yogurt bacteria and probiotic bacteria then the number of probiotic bacteria will be reduced. Hence according to the national standard No. 11325, in this study, probiotic bacteria were used as yogurt starter.

In the first hours of production, the *Bifidobacterium bifidum* yoghurt with 0.8% vanillin reached the acidity of 75°Dornic, followed by the yoghurt sample with 0.4 and 0.6% vanillin. Finally the controlled sample reached the acidity of 75°Dornic. Thus, these results indicate that vanillin has a positive effect on the growth of *Bifidobacterium bifidum* (Figure1).

The *Lactobacillus acidophilus* yoghurt with 0.8 and 0.6% vanillin reached the acidity of 75°Dornic, followed by the yoghurt sample with 0.4 % vanillin. And finally the controlled sample reached the acidity of 72°Dornic. Thus, these results indicate that vanillin has a positive effect on the growth of *Lactobacillus acidophilus*.

The mixture of *Lactobacillus acidophilus and Bifidobacterium bifidum* yoghurt with 0.8%, 0.6%, 0.4% vanillin and finally the controlled sample respectively reached the acidity of 72°Dornic which it was faster than before. These results show positive effects of vanillin on the growth of probiotic bacteria to prove it.

During the storage in the incubation, measuring the acidity and pH revealed that adding higher concentrations of vanillin to the probiotic yogurt enhances the growth of the bacteria, but no remarkable effect on their counts.

The high acidity and low pH in the probiotic fermented products is one of the most important factors responsible for the decreased survival of the probiotics. Therefore, the survival capability of these bacteria in the products such as non-sour milk is at least ten times greater than in the fermented ones and *B. bifidum* enjoys longer survival in the ice cream and yoghurt than in the milk [23].

Comparing the two passages acidity and pH produced before and after the retention period of 28 days in the fridge it was found that on average the best survival is related to before yogurt.

While After yogurt concentrations of order of 0/6, 0/4, 0/2, related strains Bifidobacterium*, Lactobacillus,* and mixed *Bifidobacterium* and *Lactobacillus*, showed the highest survival. Also highest survival in both passages related to strains of *Lactobacillus, Bifidobacterium* and mixed *Bifidobacterium* and *Lactobacillus* (Figure 2 & Figure 3).

Increasing the concentration of vanillin has a good effect on the growth of probiotics and incubation period yogurt towards control reduces, but increased vanillin concentration did not cause favorable properties and shelf life in these yoghurts, and the samples with vanillin 0.2% and the control were with the best taste, aroma, color, and thickness. There were significant differences between the controlled yoghurt on the one side and the yoghurts with 0.2%, 0.4 and 0.6% vanillin concentration with the same bacterium on the other side in terms of sensory properties and shelf life ($P<0.05$). The After yoghurts were significantly different in taste, thickness and flavor with before yoghurts ($P<0.05$), but no significant difference was detected between them.

One way to avoid decreasing the probiotic due to the increased acidity and decreased pH is to end the fermentation stage at pH level above the range of 4.7- 4.9, because the final pH of the product in such a condition during storage period reaches about 4.5. It is suggested that the final pH during storage not to be lower than 4.5.

Although, the termination of fermentation in higher pH promotes the survival of the probiotics, the taste and texture of the products would not be favorable [23, 8].

The refrigerator storage temperature directly affects the bioactivities of the probiotics through the survival of the cells in the products and indirectly affects them through the production of antimicrobial and the bio relationship created between the probiotics and the cultured bacteria during the shelf life period.

In recent years, the probiotic bacteria, as the food additives, have been introduced into numerous foods which the dairy products especially yoghurt has played an important role in carrying these bacteria [3].

Eating regularly the sufficient amounts of the living cells called "the minimum treatment' is required if the consumer is to benefit from the probiotic products. The daily recommended amount of the yogurt containing10^7 CFU.ml-1 probiotic bacteria is 100 gr. It is also very important to investigate the survival of these microorganisms within the interval between storage in the refrigerator and consumption [19]. So in this article was done by counting bacteria.

All the tested *Bifidobacterium bifidum* was capable of well growing on vanilin yoghurt without nutrient supplementation.

The sample with 0.6% had the highest bacterial counts, as the samples were evaluated by direct counting method. This results show Vanillin concentration increased effectiveness of probiotics rapid growth and increasing acidity trend and it is consistent with the Marhamatizadeh and Karmand (2011) studies, 'The increased concentration of malt and soya caused increases in the microorganism growth and acidity that in turn resulted in a shorter incubation time for the desired acidity'. In this study demonstrated that the shelf life for the acidity reaching the desired level during incubation decreased for yoghurt with both bacteria and combined soya and malt. As for the yogurt with both bacteria, the same results were obtained and incubation time for the yogurt with malt and soya was decreased. Marhamatizade and Rasekhi (2010) in their study on the effect of honey on probiotics stated that "yoghurt with only Lactobacillus acidophilus tasted more sour than the yoghurt with both bacteria. And the products containing *Bifidobacterium bifidum* were compared to those with *Lactobacillus acidophilus* and indicated that the former had slower growth rate and also tasted less sour and were longer permanence. The taste was not favorable when the concentration of honey was increased" and it was consistent with the results of our work [29]. In another study the effect of cinnamon on bacterial growth was studied and was concluded that the increased cinnamon concentration promoted the growth of the bacteria in probiotic milk and yoghurt. Further works concerned with spearmint garlic, dill extract and juices were carried out on the bacterial growth and concluded that these products promoted the growth of bacteria in probiotic milk. The effect of permeate on the growth and survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* was investigated and indicated that permeate was a suitable support for intestinal bacteria [22].

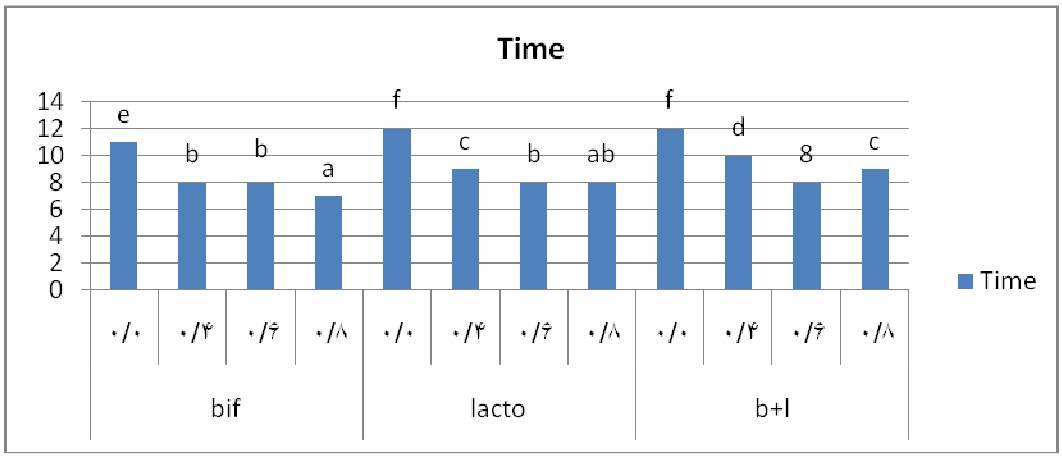

Figure 1. Chart reaching 72 degrees dornic acidity of the samples during incubation.
(Time → hours, 0/0, 0/4, 0/6, 0/8→ Percent of vanillin).

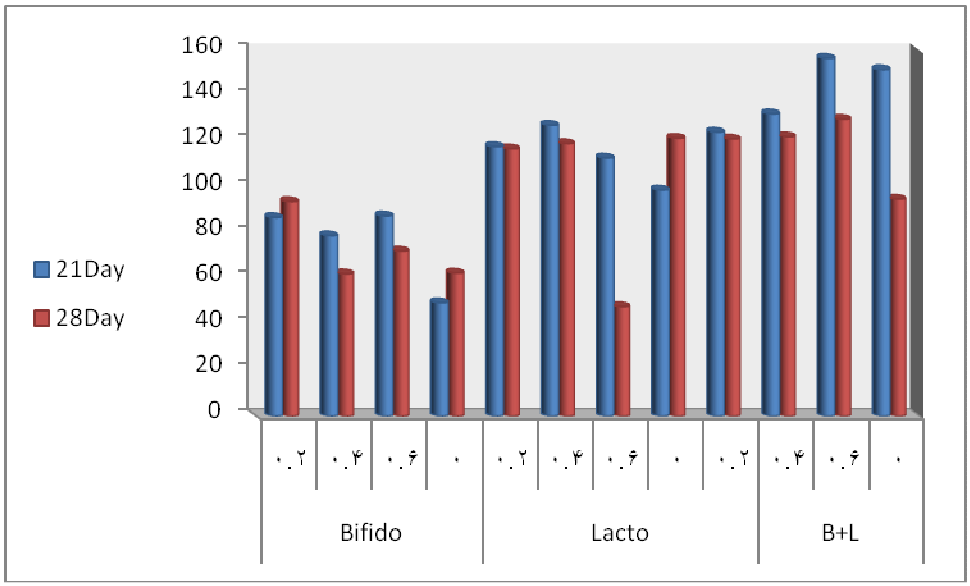

Figure 2. Acidity compared to 21 and 28 days After passage samples to determine the shelf life. (Time → hours, 0/0, 0/4, 0/6, 0/8→ percent of vanillin, Acidity→dornic).

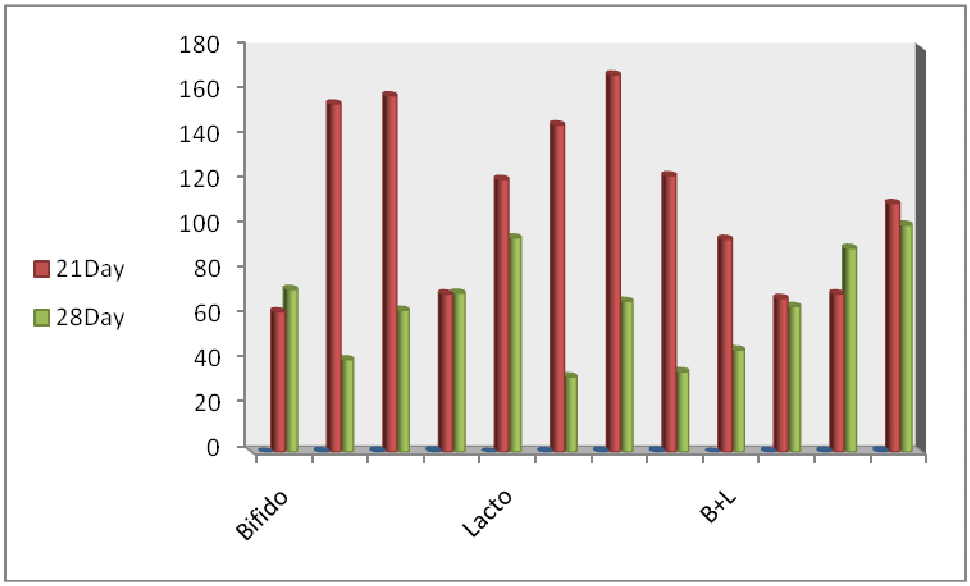

Figure 3. Acidity compared to 21 and 28 days Before passage samples to determine the shelf life. (Time → hours, 0/0, 0/4, 0/6, 0/8→ percent of vanillin, Acidity→dornic).

CONCLUSION

The present work demonstrates that the percentage of vanillin at 0.2%, 0.4%, 0.6% and 0.8% (w/v), in two passages After and Before incubation, have positive effect on fermentation and indicates the survival of probiotic bacteria in yoghurt during four-weeks storage period at 2°C. All tested strains showed a good growth rate in vanillin yoghurt without added nutrients. The decrease in the number of probiotic bacteria in vanillin yoghurt during 28 days could be avoided by strain selection and the use of greater initial inoculum levels. It is important to emphasize that all the products possessed excellent stability during 28 days of storage. It can be concluded that the addition of vanillin might causes a decrease in viscosity and consistency of the final product by reducing a protective effect on the gel-factors that it has great importance on the product acceptability. When compared with commercial yoghurt, the vanillin-flavored after yoghurt presented satisfactory rheological properties. The results might suggest that vanillin can be successfully used in formulation of dairy products. The bacterial growth in the yogurt with *B.*

bifidum was slower than other one in the products with *L. acidophilus* and tasted less sour with longer shelf life. This may be due to the low proteocaft activity of *B. bifidum* bacterium. However, when the two bacteria are present in the same products, the proteocaft activity is enhanced [23]. However, in this study, the bacterial growth in the yogurt with *B. bifidum* was faster than that one in the products with *L. acidophilus and L. acidophilus* has longer shelf life. This difference may be the effect of vanillin on improving the Bifidobacterium proteocaft activity realized. This could be the subject of future research in this area.

Acknowledgements
We thank all persons for assistance during this project.

REFERENCES

1. Akalin, A. S., S. Gonc, G. Unal & S. Fenderya, 2007. Effects of fructo oligosaccharide and whey protein concentrate on the viability of starter culture in reduced-fat probiotic yoghurt during storage: Journal of Food Science., (72): M222– M226. DOI: 10.1111/j.1750-3841.2007.00436.x.
2. Amatayakul, T., A. L. Halmos, F. Sherkat & N. P. Shah, 2006. Physical characteristics of yoghurts made using exopolysaccharide-producing starter cultures and varying casein to whey protein ratios: International Dairy Journal., (16): 40–51. DOI: 10.1016/j.idairyj.2005.01.004.
3. Cerrutti, P., S. Alzamora and S.Vidales, 1997. Vanillin as an antimicrobial for producing shelf-stable strawberry puree: Journal of Food Science., (62): 608- 610. DOI: 10.1111/j.1365-2621.1997.tb04442.x.
4. Curk, M. C., J. C. Hubert & F. Bringel, 1996. Lactobacillus paraplantarum sp. Now, a new species related to Lactobacillus plantarum: International Journal of Systematic Bacteriology., (46): 595–598.
5. Desmazeaud, M., 1996. Les bacte´ ries lactiques dans l'alimentation humaine: Utilisation etinnocuite´. Cahiers Agricultures., (5): 331–343.
6. De Vrese, M., A. Steglman, B. Richter, S. Fenselau, C. Laue & J. Scherezenmeir, 2001. Probiotics-compensation for lactase insufficiency: .American Journal of Clinical Nutrition., (73): 421–429. PMID: 11157352.
7. De Vuyst, L. & B. Degeest, 1999. Heteropolysaccharides from lactic acid bacteria: FEMS Microbiology Reviews., (23): 130–135. DOI: 10.1111/j.1574-6976.1999.tb00395.x.
8. Fulthorpe, R. and A. Grant, 1994. Evaluation of Biolog MT plates for aromatic and chloroaromatic substrateutilization tests: Canadian Journal of Microbiology., (40) 1067-1071. DOI: 1067-1071, 10.1139/m94-169.
9. Gibson, G. R., J. M. Saveedra, S. Mac- Farlane, & G. T. Mac-Farlane, 1997. Probiotics and intestinal infections. In: Fuller, R. (Ed.), Probiotic. 2: Applicationsand Practical Aspects. Chapman & Hall, New York, pp: 10–39.
10. Guler-Akin, B. & M. S. Akin, 2007. Effects of cysteine and different incubation temperatures on the microflora, chemical composition and sensory characteristics of bio-yogurt made from goat's milk: Food Chemistry 100 (2): 788–793.
11. Hong, L.H., S.M.Cutting, 2005. The use of bacterial spore formers as probiotics: FEMS Microbiology Reviews., (29):813. DOI: 10.1016/j.femsre.2004.12.001.
12. Hull, R. R., A. V. Roberts & J. J. Mayes, 1984. Survival of Lactobacillus acidophilus in yoghurt: Australian Journal of Dairy Technology.,(39): 164–166.
13. Isolauri, E., Y. Su¨ tas , P. Kankaapa, H. Arvilommi & S. Salminen, 2001. Probiotics: effects of immunity: American Journal of Clinical Nutrition., (73): 444–450. PMID: 11157355.
14. Joo, S. J., K. J. Choi, K. S. Kim, J. W. Lee & S. K. Park, 2001. Characteristics of yogurt prepared with 'jinpum' bean and sword bean (Canavalin gladiata): International Journal of Postharvest Technology and Innovation., (8): 308–312.
15. Klaver, F. A. M., F. Kingma, & A. H. Weerkamp, 1993. Growth and survival of bifidobacteria in milk: Netherlands Milk and Dairy Journal., (47): 151–164.
16. Lopez-Malo, A., S. Alzamora and A. Argaiz, 1995. Effect of natural vanillin on germination time and radial growth of moulds in fruit-basedagar systems: Food Microbiology., (12): 213- 219. DOI: 10.1007/s13197-011-0275-6.
17. Lopez-Malo, A., S. Alzamora and A. Argaiz. 1998. Vanillin and pH synergistic effects on mold growth: Journal of Food Science., (63): 143- 146. DOI: 10.1111/j.1365-2621.1998.tb15695.x.

18. Lourens Hattingh, A. & B. C. Viljoen, 2001. Yoghurt as probiotic carrier food: International Dairy Journal., (11): 1–17. DOI: 10.1016/S0958-6946(01)00036-X.
19. Marhamatizadeh, M. H., M. Mahmodi, 2009. *Study on honey yoghurt asthe bearer of Probiotic bacteria Lactobacillus acidophilus and Bifidobacteriumbifidum*: (Doctors of Veterinary Medicine thesis Islamic Azad University, Islamic Azad University of Kazerun). P, 687.
20. Marhamatizade, M. H., I. RasekhI, S. Rezazadeh, M. R. Kazemi, 2010. Study on honey yoghurt as the carrier of probiotic Bifidibacterium bifidum: *Journal of Veterinary Pathobiology*., (1): 31-40.
21. Marhamatizadeh, M. H., M. Karmand, A. R. Farokhi, R. Rafatjoo, & 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.
22. Marhamatizadeh, M. H., E. Ehsandoost, P. Gholami, H. Moshiri, & M. Nazemi, 2012. Effect of Permeate on Growth and Survival of Lactobacillus acidophilus and Bifidobacterium bifidum for Production of Probiotic Nutritive Beverages: World Applied Sciences Journal 18 (10): 1389-1393. DOI: 10.5829/idosi.wasj.2012.18.10.63242.
23. Mortazavian A. M, S. Sohrabvandi, 2006. Probiotic and Probiotic foods: Ata publish, pp. 213-264. Standard anistitue and industrial search of Iran. Milk and dairy. Finding acidity and pH-Test method, Notional industrial number 9985. DOI: 10.5897/AJFS11.138.
24. Medina, L. & R. Jordano, 1994. Survival of constitutive microflora in commercially fermented milk containing bifidobacteria during refrigerated storage: Journal of Food Protection., (56): 731–733.
25. Saarela, M., L. Lahteenmaki , R. Crittenden, S. Salminen, & T. Mattila-Sandholm, 2002. Gut bacteria and health foods: The European perspective: International Journal of Food Microbiology., (78): 99–117. DOI: 10.1016/S0168-1605(02)00235-0.
26. Shori A. B. & A. S. Baba, 2011. Antioxidant activity and inhibition of key enzymes linked to type-2 diabetes and hypertension by Azadirachta indica-yoghurt: Journal of Saudi Chemical Society. DOI: 10.1016/j.jscs.2011.04.006.
27. Tamime, A. Y., M. Saarela, A. K. Sondergaard, V. V. Mistry & N. P. Shah, 2005. Production and maintenance of viability of probiotic micro-organisms in dairy products: In A. Y. Tamime (Ed.), Probiotic dairy products (pp. 39–72). ISBN: 1-4051-2124-6.
28. Temmerman, R., B. Pot, G. Huys, & J. Swings, 2002. Identification and antibiotic susceptibility of bacterial isolates from probiotic products: International Journal of Food Microbiology., (81): 1–10. DOI: 10.1016/S0168-1605(02)00162-9.
29. Yaghtin, A. R., M. H. Marhamatizade, 2009. *The Study of Cinnamon effect on Lactobacillus acidophilus and Bifidobacterium bifidum growth in probiotic milk banana Production*. (Doctors of Veterinary Medicine thesis Islamic Azad University, Azad University of Kazerun). P, 733.
30. Zottola, E. A., T. L.Yessi, D. B. Ajao, & R. F. Roberts, 1994. Utilization of cheddar cheese containing Nisin as an antimicrobialagents in other foods: International Journal of Food Microbiology 24, 227–238. DOI: 10.1016/0168-1605(94)90121-X.