Available online at http://www.ijabbr.com

International journal of Advanced Biological and Biomedical Research

Volume 1, Issue 9, 2013: 1033-1039

The effect of *Dorema aucheri* on the activities of *bifidobacterium bifidum* and *lactobacillus acidophilus* in probiotic milk and yoghurt

Sadegh zafari [1], Mohammad Hossein Marhamatizadeh [2] and Parisa Azadneya [3]

[1] Doctor of veterinary medicine, Department of food hygiene, Veterinary faculty, Kazerun branch, Islamic azad university, Iran.

[2, 3] Assistance Professor, Department of food hygiene, Veterinary faculty, Kazerun branch, Islamic azad university, Iran.

ABSTRACT

Chicory plant (*Dorema aucheri*) belongs to Asteraceae family and contains flavonoids. Milk is a mixture of protein, fat, lactose, minerals. This research carried out in order to evaluate the effect of chicory plant on the activities of *bifidobacterium bifidum* and *lactobacillus acidophilus.* Four containers containing a liter of 1.5 % sterilized skim milk were selected as four group in order to produce milk containing probiotic *bifidobacterium bifidum*, then 0.33 gr starter of *bifidobacterium bifidum* added and after that 0 %, 0.03%, 0.06% and 0,09% the powder of *Dorema aucheri* were added. Samples were considered based on pH, acidity and microbes count. Sensory evaluation was done in fourteenth day. Results showed the maximum activity of bacteria was recorded in fourteenth day so that when this value was increased, the acidity also was being increased. Consistency time of product was determined 21 days and also bacteria indicated significant progress on MRS agar.

Key words: Probiotic, Chicory plant, Lactobacillus *acidophilus*, yoghurt, *Dorema aucheri*

INTRODUCTION

Milk is a mixture of protein, fat, lactose, minerals, etc (Jensen ,1995). The most ingredient is water with 87 % and the approximate amount in 100 grams (protein, lactose, minerals, vitamins, and aflatoxin is mentioned (Enb et al, 2009;lindmarkm, 2008). The microorganisms primarily associated with this balance are lactobacilli and bifidobacteria. Factors that negatively influence the interaction between intestinal microorganisms, such as stress and diet, lead to detrimental effects in health. Increasing evidence indicates that consumption of 'probiotic' microorganisms can help maintain such a favourable microbial profile and results in several therapeutic benefits. In recent years probiotic bacteria have increasingly been

Corresponding Author E-mail: zafarisadegh@sabamail.com

incorporated into foods as dietary adjuncts. One of the most popular dairy products for the delivery of viable *Lactobacillus acidophilus* and *Bifidobacterium bifidum* cells is bio-yogurt. Adequate numbers of viable cells, namely the 'therapeutic minimum' need to be consumed regularly for transfer of the 'probiotic' effect to consumers. Consumption should be more than 100 g per day of bio-yogurt containing more than 106 cfu mL−1 (Rybka & Kailasapathy, 1995). Functional food is the food that covers at least a distinctly and provably healthy characteristic and it is recommended by food scientists or producers as functional food. Milk and dairy products especially fermented milk products are significantly functional food. Safety in consumption is the main factor in consuming functional food. Today, there are many considerations in connection with consuming functional food that due to progress in food sciences. Probiotics are one of the newest and the most popular derivatives that are very important about this value. Functional compound of probiotic due to bacteria but other functional foods have abiotic compounds (Sarrela et *al*, 2000; Ziemer and Gibson, 1998; Mansourbahmani *et al*, 2013; Asgari safdar *et al*, 2013). Probiotics issues and their advantages have been discussed for many years. Recent studies have shown curable effects of probiotics and now they are known as healthy components. Using probiotics enable human body for combating most diseases especially intestinal diseases (Khosravi Darani and Koshki, 2008; Bayati Zadeh and Moradi kor, 2013; Sadat hoseini *et al*, 2013; Ezzati kaklar *et al*, 2013). The researcher studied the survival of Lactobacillus acidophilus and Bifidobacterium bifidum in commercial yoghurt during refrigerated storage and Three of the five products contained 107–108 g−1 viable cells of *L. acidophilus*, whereas the other two products contained 105 of this organism initially. Similarly, the initial count of *B. bifidum* was 106–107 g−1 in two of five products, while the viable numbers of this organism were 103 in the other three products. All the products showed a constant decline in the viable count of *L. acidophilus* and *B. bifidum* during storage. Initial pH values ranged from 4.07 to 4.36 and these declined after five weeks of storage to pH 3.8–4.26 (Nagendra *et al*, 1995). The researcher studied survival of Lactobacillus acidophilus and Bifidobacterium bifidum in Ice Cream for Use as a Probiotic Food and we demonstrated that probiotic ice cream is a suitable vehicle for delivering beneficial microorganisms such as *L. acidophilus* and *B. bifidum* to consumers. The bacteria can be grown to high numbers in ice cream mix and remain viable during from storage (Hekmat and McMahon, 1999).The researcher studied the survival and therapeutic potential of probiotic organisms with reference to Lactobacillus acidophilus and Bifidobacterium spp. And results showed that the inclusion of probiotic bacteria in fermented dairy products enhances their value as better therapeutic functional foods. However, insufficient viability and survival of these bacteria remain a problem in commercial food products. By selecting better functional probiotic strains and adopting improved methods to enhance survival, including the use of appropriate prebiotics and the optimal combination of probiotics and prebiotics (synbiotics), an increased delivery of viable bacteria in fermented products to the consumers can be achieved (Kailasapathy and Chin, 2000). The researcher studied the survival of probiotic microflora in Argentinian yoghurts during refrigerated storage and results showed that the culture SISD was clearly more inhibitory for both probiotic organisms than the culture SID. The loss of cell viability in yoghurt samples was different (higher in some cases and lower in others) from that due to lactic acid only. In general, pH values of 4.5 or lower jeopardised the cell viability of the probiotic organisms in yoghurt stored at 5°C. This work shows the importance of selecting a suitable combination of probiotic strains and starter cultures when different yoghurt types are formulated (Vinderola *et al*, 2000). The researcher evaluated the encapsulation techniques of probiotics for yoghurt. International Dairy Journal and emphasis the health benefits provided by probiotic bacteria have led to their increasing use in fermented

and other dairy products. However, their viability in these products is low. Encapsulation has been investigated to protect the bacteria in the product's environment and improve their survival. There are two common encapsulation techniques, namely extrusion and emulsion, to encapsulate the probiotics for their use in the fermented and other dairy products. This review evaluates the merits and limitations of these two techniques, and also discusses the supporting materials and special treatments used in encapsulation processes (Krasaekoopt *et al*, 2003). The new issues of prohibits and their aspects and complexity are the main factors that attract scientists for probiotic research. The effect of *Dorema aucheri* on improving the growth speed of *acidophilus lactobacillus* and *bifidobacterium bifidum* in producing probiotic milk and yoghurt were studied in this research.

STUDY METHOD

Four containers containing a liter of 1.5 % sterilized skim milk were selected as four group in order to produce milk containing probiotic *bifidobacterium bifidum*, then 0.33 gr starter of *bifidobacterium bifidum* added and after that 0 %, 0.03%, 0.06% and 0,09% the powder of *Dorema aucheri* were added. All samples were maintained in 38 ° C. acidity test was done for each time after about two hours to reach 42 ° D (4-7). After all samples had reached 42 ° D degree in greenhouse, they would be sent to refrigerator with 2 ° C. Probiotic milk produced was counted every five days for counting microbes based on directly counted method. A liter of sterilized skim milk and 15 gr probiotic milk of control group of firs passage were added to each container of four ones. The various concentration of *Dorema aucheri* with the amount of 0 % (control), 0.03%, 0.06% and 0,09% orderly were added to all containers, then all containers were sent to greenhouse with 38 ° C. acidity test was done for each time every two hours to reach 90 ° D. After all samples had reached 90 ° D in greenhouse, they would be sent to refrigerator with 2 ° C. Probiotic milk produced with *Dorema aucheri* was counted every seven days for counting microbes based on directly counting method and after 14 days all yoghurt samples would be evaluated base on sensory features (8). Sensory evaluation was performed by questionnaire in a population of thirty persons (9). Some components including aroma, smell, taste and consistency in four levels of very good, good, fair and poor were considered in each questionnaire. The questionnaire results were analyzed by SPSS software based on descriptive statistics. Four containers containing a liter of 1.5 % sterilized fat were selected as four groups in order to produce milk containing *lactobacillus acidophilus* (first passage) and all steps like previous one were done but in this section *lactobacillus acidophilus* was used instead of *bifidobacterium bifidum* and also for producing yoghurt containing *lactobacillus acidophilus* (second passage) and all steps like previous one were done but in this section *lactobacillus acidophilus* was used instead of *bifidobacterium bifidum.*

RESULTS AND DISCUSSION

Samples of five brands of commercial yoghurt were obtained directly from the processors and enumeration of viable *Lactobacillus acidophilus* and *Bifidobacterium bifidum* and determination of pH was carried out at three-day intervals over a five-week period (Nagendra *et al*, 1995). Milk and yoghurt acidity of probiotic chicory have been shown in table 1. The therapeutic potential of these bacteria in fermented dairy products is dependent on their survival during manufacture and storage. Probiotic bacteria are increasingly used in food and pharmaceutical applications to balance disturbed intestinal microflora and related dysfunction of the human gastrointestinal tract. Lactobacillus acidophilus and

Bifidobacterium spp. have been reported to be beneficial probiotic organisms that provide excellent therapeutic benefits. The biological activity of probiotic bacteria is due in part to their ability to attach to enterocytes (Kailasapathy and Chin, 2000). In this research, the effect of chicory on *bifidobacterium bifidum and* lactobacillus *acidophilus* and the possibility of producing a newly probiotic product based on milk on chicory were evaluated. The variations of chicory milk and yoghurt containing *bifidobacterium bifidum and lactobacillus acidophilus* regarding to acidity, pH and viability of probiotic bacteria in two-hour intervals to reach acidity 42 ° D of milk and 90 ° D of yoghurt in the 38 ° C greenhouse and 21 days keeping in fridge were recorded. Acidity values in early hours were constant and this value due to not starting probiotic activities. Samples containing probiotic yoghurt to reach 90 ° D were sent to the 38 ° C greenhouse that in early hours, pH values were closes each other but after about 8 hours they had been soddenly increased and became more than 90 ° D. The control sample (0%) of *bifidobacterium bifidum* and 0.09% of *lactobacillus acidophilus* had been reached favorite acidity sooner than others, then they were sent to fridge. *lactobacillus acidophilus* has less the incubation time than *bifidobacterium bifidum.* While medicinal properties of probiotic productions are the main character of them but their sensory properties have an important level in food sciences. Among probiotic products, fermented products especially probiotic yoghurt due to unique properties are popular in the world. Probiotic yoghurt can be considered as the most important probiotic product (10 and 11).The various groups of 0 %, 0.03%, 0.06% and 0.09% regarding to smell and aroma, color, consistency, flavor, fat and taste were studied. For analyzing these properties non-parametric methods were used that there were not found significant differences among all samples. There were not favorite flavor in yoghurt by increasing chicory in samples containing *lactobacillus acidophilus* or *bifidobacterium bifidum* separately and in samples containing both *lactobacillus acidophilus* and *bifidobacterium bifidu* and also yoghurt with 0.03% and 0% chicory had the best favorite flavor among all samples.Increasing chicory concentration didn't have any effect on yoghurt consistency and yoghurt containing 0.03% and 0% chicory had more favorite flavor than others and also yoghurt containing 0.03% chicory had more favorite color and smell than other samples. Products containing *bifidobacterium bifidum* had slower growth and sweeter as well as longer consistency than those containing *lactobacillus acidophilus.* Probiotic yoghurts felt fatty while skim milk was used for producing products. Despite significant progresses in probiotic field, there is not a unique and global criterion for this value but some countries such as Japan has a distinct and significant criterion for this issue (12). The number of probiotic live cells in each gram of products shows the essential value of probiotic products. This value as an indicator determines the efficiency of these products. Cited indicator due to biological value and its minimum called minimum biological value (MBV). The most important baseline in connection with MBV indicator of probiotic products by IDF is $١٠^{٧}$(13 and 14). Microbial count during incubation and product consistency to determine the growth rate of bacteria was done based on direct count. Results showed the number of starter bacteria except in *bifidobacterium bifidum* after 21 days were significantly decreased. There was not found significant difference between control and samples containing various concentrations of chicory. This means that concentration of chicory didn't have significant effect on starter bacteria. *Bifidobacterium bifidum* samples of yoghurt and milk were increased during 21 days. The concentration of probiotic bacteria for showing their properties in consumer body should be 10^7 per gram. In this research the amount of microbes was 10^7, so that desired properties of probiotic microbes were created in consumer.

Conclusion

Some studies carried out on malt, soy, honey, cinnamon and chicory. Comparing results showed that probiotic chicory caused to increase growth rate of *bifidobacterium bifidum* and *lactobacillus acidophilus* in dairy products.

REFERENCES

Bayati Zadeh. J, Moradi kor. Z. (2013). Ruminal protein degradation and estimation of rumen microbial protein production. International journal of Advanced Biological and Biomedical Research (IJABBR) . 1(8):914-921.

Ebrahimpour. M., Kiyani. V., Mohammadhasan. H. (2013). Investigation acute toxicity some of heavy metals at different water hardness. International journal of Advanced Biological and Biomedical Research (IJABBR) . 1(2):134-142.

Enb,A.,AbouDonia,M.,Abd-Rabou,N.,Abou-Arab,A.,El-Senaity,M(2009).Chemical Composition of Raw Milk and Heavy Metals Behavior During Processing of Milk Products. Global Veterinaria. 3 (3): 268- 275.

Ezzati kaklar. A., Moradi kor. N. (2013). The effects of psychological stress and other environmental factors on incidence of diseases. International journal of Advanced Biological and Biomedical Research (IJABBR) . 1(8):899-903.

Farkhonde. A. (1998). *Test method of milk and dairy*. Forth adition. Tehran University publishes. 266-270.

Hekmat. S., McMahon. D. (1999). Survival of Lactobacillus acidophilus and Bifidobacterium bifidum in Ice Cream for Use as a Probiotic Food. Journal of Dairy Science. 75(6): 1415–1422.

Jensen,R. (1995). Handbook of milk composition. New York, NY: Academic Press.

Kailasapathy. K., Chin. J. (2000). Survival and therapeutic potential of probiotic organisms with reference to Lactobacillus acidophilus and Bifidobacterium spp. mmunology and Cell Biology (2000) 78, 80–88.

KhosraviDarani. K., Koshki, MR.*(2008). Probiotics in the milk and dairy*. Marzedaneshpublish, Firstadition, 243-244.

Krasaekoopt. W., Bhandari. B., Deeth. H., 2003. Evaluation of encapsulation techniques of probiotics for yoghurt. International Dairy Journal, 13(1): 3–13.

lindmarkm, H.(2008)Fatty acids in bovine milk fat. Food Nutr. Res. 52.

Mansourbahmani. M., Heravi Moussavi. A., Vakili.A., Rezaie. M.R., Dehghan. H.R., Rezvan Najad. E. (2013). Study of Existing Radon In Milk and Its Effect on Body Organs. International journal of Advanced Biological and Biomedical Research (IJABBR) . 2013; 1(8):802-812.

Marhamatizadeh, MH., Kazeroonian, H. *(2009). Study on honey yoghurt as the bearer of Probiotic bacteria's Lactobacillus acidophilus.* Doctors of Veterinary Medicine thesis Islamic Azad University. Number 689, Azad University of Kazerun.

Marhamatizadeh, MH., Rafatjoo, R., Farokhi. AR., Karmand. M., Rezaazade, S. (2009). *The study of soya extracts on the growth of probiotic Lactobacillus acidophilus and Bifidobacerium bifidum bacteria in probiotic milk and yoghurt.* Journal of Veterinary Pathobiology 1: 23-28.

Marhamatizadeh. MH., Mahmodi, M. (2009). *Study on honey yoghurt as the bearer of Probiotic bacteria Lactobacillus acidophilus and Bifidobacterium bifidum.* Doctors of Veterinary Medicine thesis Islamic Azad University, Number 687, Islamic Azad University of Kazerun.

Mortazavian. AM., Sohrabvandi, S. (2006). *Probiotic and Probiotic foods.* Ata publish. 213-264.

Nabinejad. A. (2013). Study on subclinical and clinical Marek's disease (MD) in the broiler chickens using histopathology. International journal of Advanced Biological and Biomedical Research (IJABBR) . 2013; 1(8):795-801.

Nabinejad. A. (2013). Study on subclinical and clinical Marek's disease (MD) in the broiler chickens using histopathology. International journal of Advanced Biological and Biomedical Research (IJABBR) . 2013; 1(8):795-801.

Nagendra P. Shah, Warnakulasuriya E.V, Lankaputhra, Margaret L. Britz, William S.A. Kyle. (1995). Survival of Lactobacillus acidophilus and Bifidobacterium bifidum in commercial yoghurt during refrigerated storage. International Dairy Journal, 5(5): 515–521.

S Rybka, K Kailasapathy. 1995. The survival of culture bacteria in fresh and freeze-dried AB yoghurts. The Australian Journal of Dairy Technology, 50 (2): 51–57.

Sarrela, M., Mogensen, G., Fonden, R., Sandholm. TM.(2000). *Probiotic bacteria: safety, functional and technological properties. Journal of Biotechnology.* 84: 197-215.

Sarrela. M., Mogensen .G., Fonden .R., Sandholm. TM. (2000). *Probiotic bacteria: safety, functional and technological properties. Journal of Biotechnology,* 84: 197-215.

Standard anistitue and industrial search of Iran. (1999). Sense Test of milk and dairy with graded method. Nutionalindasterial number 781p.

Standard anistitue and industrial search of Iran. (2006). *Milk and dairy.* Finding acidity and pH-Test method. Nutionalindasterial number 9985.

Vinderola. C.G., Bailo. N., Reinheimer. J.A, 2000. Survival of probiotic microflora in Argentinian yoghurts during refrigerated storage, Food Research International, 33(2): 97–102.

Ziemer. CJ., Gibson. GR. (1998). *An overview of probiotics, orebiotics and synbiotics in the Functional food concept*: perspectives and future strategies. Int. Dairy Journal, 8: 473-479.

Table 1: The acidity of probiotic chicory milk and yoghurt containing *lactobacillus acidophilus* in 21 days in fridge.

Direct counting of bacteria in milk containing *lactobacillus acidophilus*								
21st day		14th day		Seventh day		Second day		Product
yoghurt	milk	Yoghurt	milk	yoghurt	milk	Yoghurt	milk	
92	48	94	51	89	49	84	47	0%
98	56	100	62	91	59	86	55	0.03%
103	60	108	65	94	61	90	57	0.06%
100	58	105	63	92	60	87	56	0.09%

Table 2: The acidity of probiotic chicory milk and yoghurt containing *bifidobacterium bifidum* during 21 days has been shown in table 2.

Direct counting of bacteria in milk containing *bifidobacterium bifidum*								
21st day		14th day		Seventh day		Second day		Product
yoghurt	milk	Yoghurt	milk	yoghurt	milk	Yoghurt	milk	
96	44	98	49	94	45	90	43	0%
102	56	105	60	100	57	96	54	0.03%
113	58	115	63	109	60	105	56	0.06%
107	57	110	61	105	58	100	55	0.09%

Table 3: The study of growing microbes in probiotic chicory yoghurt containing *bifidobacterium bifidum* and *lactobacillus acidophilus* separately.

Direct counting of bacteria in milk containing *lactobacillus acidophilus*								
21st day		14th day		Seventh day		Second day		Product
yoghurt	milk	Yoghurt	milk	yoghurt	milk	Yoghurt	milk	
$55*10^9$	$55*10^9$	$75*10^9$	$7*10^{10}$	$55*10^9$	$53*10^9$	$5*10^{10}$	$43*10^9$	0%
$65*10^9$	$63*10^9$	$9*10^{10}$	$8*10^{10}$	$7*10^{10}$	$65*10^9$	$63*10^9$	$5*10^{10}$	0.03%
$73*10^9$	$9*10^{10}$	$11*10^{10}$	$95*10^9$	$85*10^9$	$85*10^9$	$75*10^9$	$65*10^9$	0.06%
$75*10^9$	$8*10^{10}$	$1*10^{11}$	$85*10^9$	$75*10^9$	$78*10^9$	$7*10^{10}$	$6*10^{10}$	0.09%
Direct counting of bacteria in milk containing *bifidobacterium bifidum*								
21st day		14th day		Seventh day		Second day		Product
yoghurt	milk	Yoghurt	milk	yoghurt	milk	Yoghurt	milk	
$63*10^9$	$5*10^{10}$	$8*10^{10}$	$65*10^9$	$6*10^{10}$	$5*10^{10}$	$55*10^9$	$35*10^9$	0%
$85*10^9$	$65*10^9$	$95*10^9$	$7*10^{10}$	$83*10^9$	$55*10^9$	$7*10^{10}$	$4*10^{10}$	0.03%
$95*10^9$	$8*10^{10}$	$1*10^{11}$	$85*10^9$	$9*10^{10}$	$7*10^{10}$	$85*10^9$	$65*10^9$	0.06%
$85*10^9$	$75*10^9$	$9*10^{10}$	$8*10^{10}$	$8*10^{10}$	$75*10^9$	$83*10^9$	$55*10^9$	0.09%