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**International journal of Advanced Biological and Biomedical Research**

Volume 1, Issue 9, 2013: 1016-1021

# The effect of *Cichorium intybus* on the activities of *bifidobacterium bifidum* and *lactobacillus acidophilus* in probiotic milk and yoghurt

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## ABSTRACT

Chicory plant (*Cichorium intybus*) belongs to Asteraceae family and contains flavonoids. 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 *Cichorium intybus* were added. Samples were considered based on pH, acidity and microbes count. Sensory evaluation was done in fourteenth day. The questionnaire results were analyzed by SPSS software based on descriptive statistics. The viability of probiotic bacteria were considered based on direct count. 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, Bifidobacterium bifidum, Lactobacillus acidophilus, Milk, Cichorium intybus

## INTRODUCTION

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;

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Sadat hoseini *et al*, 2013; Ezzati kaklar *et al*, 2013). The new issues of prohibits and their aspects and complexity are the main factors that attract scientists for probiotic research. The effect of *Cichorium intybus* 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 *Cichorium intybus* 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 *Cichorium intybus* 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 *Cichorium intybus* 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**

Milk and yoghurt acidity of probiotic chicory have been shown in table 1.

**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 | |
| 86 | 46 | 94 | 49 | 89 | 47 | 84 | 45 | 0% |
| 95 | 69 | 105 | 73 | 100 | 70 | 90 | 52 | 0.03% |
| 107 | 70 | 120 | 77 | 112 | 72 | 102 | 55 | 0.06% |
| 100 | 73 | 111 | 80 | 106 | 76 | 95 | 56 | 0.09% |

The acidity of probiotic chicory milk and yoghurt containing *bifidobacterium bifidum* has been shown in table 2.

**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 | |
| 85 | 44 | 91 | 50 | 87 | 46 | 83 | 43 | 0% |
| 92 | 56 | 102 | 65 | 94 | 58 | 88 | 47 | 0.03% |
| 104 | 61 | 115 | 69 | 108 | 63 | 100 | 50 | 0.06% |
| 98 | 63 | 108 | 74 | 100 | 65 | 96 | 52 | 0.09% |

Table 3 has shown growing microbes in probiotic chicory yoghurt containing *bifidobacterium bifidum* and *lactobacillus acidophilus* separately.

**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 | |
| $55*10^9$ | $65*10^9$ | $6*10^{10}$ | $73*10^9$ | $5*10^{10}$ | $63*10^9$ | $65*10^9$ | $55*10^9$ | 0% |
| $95*10^9$ | $75*10^9$ | $82*10^9$ | $75*10^9$ | $7*10^{10}$ | $7*10^{10}$ | $67*10^9$ | $63*10^9$ | 0.03% |
| $7*10^{10}$ | $7*10^{10}$ | $1*10^{11}$ | $83*10^9$ | $9*10^{10}$ | $75*10^9$ | $8*10^{10}$ | $7*10^{10}$ | 0.06% |
| $86*10^9$ | $75*10^{10}$ | $9*10^9$ | $9*10^{10}$ | $95*10^9$ | $8*10^{10}$ | $85*10^9$ | $73*10^9$ | 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 | |
| $45*10^9$ | $53*10^9$ | $5*10^{10}$ | $55*10^9$ | $4*10^{10}$ | $5*10^{10}$ | $55*10^9$ | $45*10^9$ | 0% |
| $65*10^9$ | $53*10^9$ | $75*10^9$ | $65*10^9$ | $5*10^{10}$ | $55*10^9$ | $63*10^9$ | $5*10^{10}$ | 0.03% |
| $85*10^9$ | $6*10^9$ | $9*10^{10}$ | $7*10^{10}$ | $8*10^{10}$ | $63*10^9$ | $7*10^{10}$ | $55*10^9$ | 0.06% |
| $83*10^9$ | $73*10^9$ | $8*10^{10}$ | $75*10^9$ | $85*10^9$ | $7*10^{10}$ | $75*10^9$ | $6*10^{10}$ | 0.09% |

**Discussion**

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 $۱۰^{۷}$(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 $۱۰^{۷}$per gram. In this research the amount of microbes was $۱۰^{۷}$, 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.

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