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Research Article

STUDYING THE INFLUENCE OF ALOE VERA ON THE GROWTH OF THE LACTOBACILLUS ACIDOPHILUS AND BIFIDOBACTERIUM BIFIDUM PROBIOTIC BACTERIAS IN PRODUCING PROBIOTIC MILK AND YOGHURT

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ABSTRACT

Probiotic is a Latin term which means for life (life-giving). Probiotics are usually one or a mixture of microorganisms which consist of useful bacterias and yeasts. If consumed by humans or animals, Probiotics can have various useful benefits through improving the host's digestion microflora features. To determine the influence of various dozes Aloe Vera on the growth of Lactobacillus acidophilus and Bifidobacterium Bifidum probiotic bacterias in the first (milk) and second (yoghurt) probiotic stage, 0.33 gram of Lactobacillus acidophilus and Bifidobacterium Bifidum Lyophilized bacteria were added separately to 1 liter of low-fat milk, and the samples were studied based on their PH and their acidity during incubation and persistence (21 days) time. Observations indicated that 3% Aloe Vera probiotic milk and yoghurt with Bifidobacterium Bifidum and 2% probiotic milk and 3% Aloe Vera yoghurt in the sample which contained Lactobacillus acidophilus yielded acidity much faster than other samples. Thus, it may be concluded that increasing the density of Aloe Vera has a positive influence on the growth of Lactobacillus acidophilus and Bifidobacterium Bifidum bacteria in probiotic milk and yoghurt.

Keywords: Aloe Vera, Probiotic, Lactobacillus acidophilus, Bifidobacterium Bifidum.

INTRODUCTION

Probiotic comes from the Greek term probios (life-giving) and it is used in contrast to antibiotic. World Health Organization uses this word to indicate live organisms which will be useful for their host if used in an appropriate amount^{1,2}.

This word has apparently been used for the first time by someone named Virgio. In his notes, he has explored the harmful effects of using antibiotics on the microbial population of the intestine and made it clear that Probiotics have positive influences on this microbial flora^{3,4}.

In 1989, Fuller defined probiotics as nutritional supplements of live microbes that create positive influences in the body of their host through the balance of intestine's micro flora. This definition only recognizes the positive effects of Probiotics just through intestine's micro flora^{3,5}.

Generally, one of the nutrition groups which can be enriched very well by Probiotic bacteria is milk and yoghurt. The commonest cultivations which are used in producing these products consist of Lactobacillus acidophilus, Bifidobacterium Bifidum, moratory and makazei. As soon as these bacteria enter the digestion system, they balance and improve the useful bacteria in the intestine¹.

Herbal medicines are used with various solutions in all cultures. The zenith of using herbal medicines was in late 1800s and early 1900s. In fact, herbal medicines play an important role in curing and preventing disabling disease in people. Aloe Vera is an important vital herb which is used for various purposes such as burning, boil, injuries and various other treatments. Papyruses of the ancient Mesopotamian Egypt used clay tablets containing Aloe Vera as an active element fir curing infection and skin problems as a laxative.

The Chinese also used Aloe Vera for curing cold and skin problems due to her concentration of cooling gel⁶.

METHODOLOGY

To study the influence of Aloe Vera in producing Bifidobacterium Bifidum probiotic milk, we provided four containers containing 1 liter low-fat, sterilized milk with 1.5% fat. Then some 0.33 gram of starter (Bifidobacterium Bifidum) was added. Next in the order of percentage (witness), 1%, 2%, 3% (1, 2, 3 gram) Aloe Vera was added to the containers. The containers were incubated in 38 degrees centigrade. Acidity and PH tests were conducted on all the dishes every 2 hours until they reached dornic 42-degree acidity. After reaching the preferred acidity, the samples were taken out of the incubator to a refrigerator with a temperature of 2 degrees centigrade. (The acidity of the milk is mostly reported based on the lactic acid and dornic degree. One degree of dornic equals 0.1 gram of lactic acid in one liter milk. The acidity of the fresh milk is 0.14 to 0.15 percent per lactic acid or 14-15 dornic degrees. The difference in measuring the acidity of milk based on the percentage of lactic acid and the dornic degree is that the normality of the consumed profit in the dornic method is N/9, while acidity based on lactic acid uses a normality value of N/10)^{7-9,5}.

In the second treatment for studying the influence of Aloe Vera in producing Bifidobacterium Bifidum probiotic yoghurt in the second passage, 1 liter of sterilized low-fat milk and 15 grams (1.5%) probiotic milk of the witness group from the first passage and 15 grams (1.5%) low-fat rennet were added to the 4 containers. Then, various densities of Aloe Vera (0%, 1%, 2%, 3%) were added respectively to all containers;

afterwards they were stirred so that Aloe Vera got dissolved evenly in the container. All the containers were placed in an incubator with a temperature of 38 degrees centigrade. The acidity and PH tests were conducted every 2 hours till they reached 70-degrees dornic acidity. After they had reached the preferred acidity, they were moved to a refrigerator with a temperature of 2 degrees centigrade. The resulting probiotic Aloe Vera yoghurt was evaluated on the fifth day for counting microbes in direct method. After 10 days, the yoghurts were measured for their sensory characteristics^{7,9}.

The study of the influence of Aloe Vera in producing Lactobacillus acidophilus probiotic milk and yoghurt is also similar to probiotic milk which has Bifidobacterium Bifidum. The only difference is that Lactobacillus an acidophilus bacterium is used instead of Lyophilized Bifidobacterium Bifidum bacteria¹⁰.

RESULTS AND DISCUSSION

Table 1 shows the acidity level based on the dornic degree in Lactobacillus acidophilus probiotic milk during the incubation process. Table 2 shows the acidity level based on the dornic degree in Bifidobacterium Bifidum probiotic milk during the incubation process. Table 3 shows the acidity level based on the dornic degree in Lactobacillus acidophilus probiotic yoghurt, and table 4 shows the acidity level based on the dornic degree in Bifidobacterium Bifidum probiotic yoghurt during the incubation process. Tables 5 and 6 show the growth rate of microbes in Lactobacillus acidophilus probiotic yoghurt and milk. Tables 7 and 8 show the growth rate of microbes in Bifidobacterium Bifidum probiotic yoghurt and milk.

Table -1- Acidity in Lactobacillus acidophilus milk

The acidity level based on the dornic degree during the incubation process (hours)				
Time	Witness	1%	2%	3%
00:00	20	20	20	20
02:00	20	20	21	22
04:00	23	24	24	26
06:00	32	28	27	28
08:00	38	32	32	32
09:00	38	35	36	35
11:15	41	42	42	41
11:50	42	-	-	42

Table-2- Acidity in Bifidobacterium Bifidum milk

The acidity level based on the dornic degree during the incubation process (hours)				
Time	Witness	1%	2%	3%
00:00	19	19	20	19
02:00	22	23	23	23
04:00	28	26	25	27
07:00	36	34	35	36
08:00	39	37	37	42
10:00	42	42	40	-
12:00	-	-	42	-

Table-3- Acidity in Lactobacillus acidophilus yoghurt

The acidity level based on the dornic degree during the incubation process (hours)				
Time	Witness	1%	2%	3%
00:00	21	21	22	21
02:00	22	24	24	22
04:00	31	32	34	43
05:40	42	47	53	55
06:35	53	52	55	62
06:50	54	58	68	70
07:15	59	70	70	-
07:30	70	-	-	-

Table-4- Acidity in Bifidobacterium Bifidum yoghurt

The acidity level based on the dornic degree during the incubation process (hours)				
Time	Witness	1%	2%	3%
00:00	21	21	20	21
02:00	22	24	24	22
04:00	31	32	34	43
03:05	42	47	53	55
04:05	43	52	55	62
05:15	54	58	68	70
05:50	59	70	70	-
06:00	70	-	-	-

Table-5- Microbial population in Lactobacillus acidophilus yoghurt

Microbial population cfu/g	First day	Fifteenth day
Witness	6.25×10^{10}	7.75×10^{10}
1% Aloe Vera	8×10^{10}	4.75×10^{10}
2% Aloe Vera	6.25×10^{10}	6.50×10^{10}
3% Aloe Vera	12.25×10^{10}	7.25×10^{10}

Table-6- Microbial population in Lactobacillus acidophilus milk

Microbial population cfu/g	First day	Fifteenth day
Witness	14.25×10^{10}	10.75×10^{10}
1% Aloe Vera	12×10^{10}	8×10^{10}
2% Aloe Vera	13×10^{10}	9.5×10^{10}
3% Aloe Vera	11.75×10^{10}	7.25×10^{10}

Table-7- Microbial population in Bifidobacterium Bifidum yoghurt

Microbial population cfu/g	First day	Fifteenth day
Witness	8.5×10^{10}	2.75×10^{10}
1% Aloe Vera	7×10^{10}	3.5×10^{10}
2% Aloe Vera	6.25×10^{10}	6.75×10^{10}
3% Aloe Vera	6×10^{10}	6.25×10^{10}

Table-8- Microbial population in Bifidobacterium Bifidum milk

Microbial population cfu/g	First day	Fifteenth day
Witness	17.75×10^{10}	13.25×10^{10}
1% Aloe Vera	11.5×10^{10}	6.75×10^{10}
2% Aloe Vera	6.75×10^{10}	5.5×10^{10}
3% Aloe Vera	6.25×10^{10}	4.75×10^{10}

In recent years, Probiotic bacteria as food additives have been mixed with various foods, among which dairy especially yoghurt play a major role in carrying these bacteria (like *Bifidobacterium Bifidum* and *Lactobacillus acidophilus*). Environments with the base of milk or the conventional whey are suitable environments for cultivating and reproducing Probiotics. Generally, milk is a suitable environment for microbes to cultivate. It contains nutrients (Carbohydrates, fat, protein, vitamin and minerals), high free water, nearly neutral PH and, unlike other solid nutrients, it does not have fiber, protein, and cellular guards which are digested late, hard to digest or indigestible^{3, 11, 12}. Aloe Vera as a medical herb has a lot of attributes some of which are anti-allergy, anti-inflammation, laxative, anti-blood pressure, empowering the immune system, aging skin prevention, and reduction of blood fat⁶.

In this research, we have set out to study the influence of Aloe Vera on the growth of *Lactobacillus acidophilus* and *Bifidobacterium Bifidum* probiotic bacteria and the possibility of producing a new Probiotic food product based on milk and Aloe Vera.

The results of producing *Bifidobacterium Bifidum* Probiotic milk and Aloe Vera shows that 3% Aloe Vera Probiotic milk in the sample containing *Bifidobacterium Bifidum* reached 42-dornic-degree acidity sooner than the other samples. The witness and 1% Aloe Vera samples had a positive influence on the growth of *Bifidobacterium Bifidum* bacteria in Probiotic milk, and the level of resulting lactic acid increased. Finally, it reached the preferred acidity sooner than other samples.

Studying the results of producing *Bifidobacterium Bifidum* Probiotic yoghurt and Aloe Vera, the following conclusion was drawn that 3% Aloe Vera Probiotic yoghurt in the sample containing *Bifidobacterium Bifidum* reached the 70-dornic-degree acidity, and afterwards samples with 1% Aloe Vera and 2% Aloe Vera and, finally, witness sample reached the preferred acidity level respectively. Next, they were transferred to a refrigerator with the temperature of 2 degrees centigrade. Given that the witness sample contained just bacteria and no amount of Aloe Vera is added to it and that 3% Aloe Vera sample reached the preferred acidity level earlier than the witness sample, it can be concluded that 3% Aloe Vera has had a positive influence on the growth of bacteria and helped reach the preferred acidity level earlier.

The results of producing *Lactobacillus acidophilus* Probiotic milk and Aloe Vera shows that 2% Aloe Vera Probiotic milk in the sample containing *Lactobacillus acidophilus* reached 42-dornic-degree acidity sooner than the other samples. Afterwards, 1% Aloe Vera sample and 3% Aloe Vera sample and, finally, the witness sample reached 42-dornic-degree acidity respectively. Thus, 2% Aloe Vera level has had a positive effect on the growth of *Lactobacillus acidophilus* bacteria in Probiotic milk.

The results of producing *Lactobacillus acidophilus* Probiotic yoghurt and Aloe Vera shows that 3% Aloe Vera Probiotic milk in the sample containing *Lactobacillus acidophilus* reached 70-dornic-degree acidity sooner than the other samples. Afterwards, 2% Aloe Vera sample and 1% Aloe Vera sample and, finally, the witness sample reached 70-dornic-degree acidity respectively.

As for direct counting of the microbes, although the counted bacteria in all densities of Aloe Vera and witness sample still had the logarithmic Coefficient 10^{10} , the number of microbes in the 15th day had decreased compared to the 1st day. In the sensory measurement of the yoghurt samples produced, it was observed that increasing the density of Aloe Vera improves the consolidation of yoghurt and also gives a better taste to yoghurt.

Finally, we can conclude that increasing the level of Aloe Vera in probiotic milk and yoghurt samples increases the growth rate of probiotic bacteria used in them.

Studying the results and comparing other theses on probiotic bacteria in Kazeroon Faculty of Veterinary Medicine, the following conclusions were drawn:

1- Increasing the density of malt and soybean improved the growth rate of the microbes, and the acidity growth pace increased. As the density increased, the incubation time to reach the preferred acidity decreased⁷.

2- In a study conducted about the influence of soybean powder on growth of *Lactobacillus acidophilus* and *Bifidobacterium Bifidum* bacteria in producing Probiotic milk and yoghurt, it was observed that incubation time to get to the preferred acidity level while producing *Lactobacillus acidophilus* and *Bifidobacterium Bifidum* milk was much less than when malt and soybean extracts are used simultaneously to produce milk. It was further observed that incubation time was much less while producing *Lactobacillus acidophilus* and *Bifidobacterium Bifidum* yoghurt using malt and soybean extract than when soybean is used to produce Probiotic yoghurt^{7,9}.

3- In a study conducted about the influence of honey on increasing the simultaneous growth rate of *Lactobacillus acidophilus* and *Bifidobacterium Bifidum* Probiotic bacteria in dairy and drinking products, it was observed that *Lactobacillus acidophilus* and *Bifidobacterium Bifidum* yoghurt was sourer than the yoghurt which contained *Lactobacillus acidophilus* and *Bifidobacterium Bifidum* microbes. Products containing *Bifidobacterium Bifidum* had a slower growth rate than those containing *Lactobacillus acidophilus*. The former products were sweeter and had longer durability⁸.

Swenson (1999) stated that Probiotics are capable of growth during the fermentation period. This leads to a general increase in the number of bacteria and decreases the final price of the product. On the other hand, it causes the bacteria to get used to the product environment more than ever.

Yoghurt is a relatively cheap food product which is used by the majority of the people. People who are not able to use raw milk are recommended to replace it with yoghurt^{13, 14}. Given the public consumption of this nutrient substance and the profits of Probiotic microorganisms in humans' health and good growth and durability of this microorganism in dairy products and the relatively quick growth of Probiotic dairy products consumption in the developed and industrial countries, it is reasonable to consider this matter in Iran, too.

CONCLUSION

Based on the results of this research, the following recommendations are put forward¹⁵:

1- Probiotic Aloe Vera yoghurt should be produced in factories and supplied in markets.

2- It is better to use 3% density Aloe Vera to produce Lactobacillus acidophilus Probiotic Aloe Vera yoghurt.

3- It is better to use 3% density Aloe Vera to produce Bifidobacterium Bifidum Probiotic Aloe Vera yoghurt.

REFERENCES

1. Khosravi Darani Kianoush and Koushki Mohammad Reza. probiotics in milk and dairy products, Marzedanesh Press, first edition, 2008; pp. 12-1.
2. Mirzaei Hamid and Karim Giti. the possibility of producing a mineral supplement probiotic cultures Lactobacillus Casei, Journal of Veterinary Science. 2004; pp. 76 to 88.
3. Mortazavian, Amir Mohammad, Sohrabvandi Sara. An overview of probiotics and probiotic food products, Eta publications, First Edition, 2006.
4. Spanhaak S, Havenaar R. and Schaafsma G. European Journal of clinical Nutrition. 1998; 52, 899.
5. Marhamati Zadeh, Mohammad Hossein, Abbasi Mohammad Ali. Study of thyme effect on bacteria Lactobacillus acidophilus and bifidobacterium bifidum milk, yogurt, probiotics, professional veterinary doctoral thesis, No. 694, Islamic Azad University of Kazeroon Branch, 2009.
6. Geffrey bland. effect of orally consumed Aloe vera Juice.1985;7(7), 10.
7. Marhamati Zadeh, Mohammad Hossein, Farokhi Alireza. Study of simultaneous effect of malt extract and soybean on growth of lactobacilli probiotic bacteria Bacillus acidophilus and bifidobacterium bifidum in probiotic yogurt, milk production and

professional veterinary doctoral thesis, Islamic Azad University of Kazeroon Branch, 2008.

8. Marhamati Zadeh, Mohammad Hossein, Rasekh Iman. Evaluation of honey yogurt lactobacilli as carriers of probiotic bacteria Bacillus acidophilus, professional veterinary doctoral thesis, No. 689, Islamic Azad University of Kazeroon Branch, 2009.
9. Marhamati Zadeh, Mohammad Hossein, Rafatjou Reza. Study of effect on the growth of probiotic bacteria Lactobacillus acidophilus and bifidobacterium bifidum soy milk and yogurt, probiotics, professional veterinary doctoral thesis, Islamic Azad University of Kazeroon Branch, 2008.
10. Institute of Standards and Industrial Research of Iran. milk and dairy products, Test Method for Determination of acidity and PH, 9985 national standard, 2005.
11. Holzapfel WH and Steel JL. probiotic bacteriu, Safety, functional and technological properties. Journal of Biotechnology. 2001; 35-109.
12. probiotics and human health:a clinical persective postgard med j.2006.
13. Koushki MR. Shadnia FZh, Raeisi A, Mohsenian Abyaneh M. examine the strategies and challenges of probiotic yoghurt production (Urals), the Sixteenth National Congress of Food Technology, safety, waste reduction and Innovation, University of Agriculture and natural Resources, Gorgan, Iran, 2005.
14. Swensen U. probiotics: Acritica review. Horizon scientific press, wymondham, 1999; 57-64.
15. Roisin Hughes, Daniel Commune, Reasearch 591. Available online at www. Science direct. Com. 2005; 276-278.

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