ISSN : 0974 - 7435 Volume 9 Issue 10

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 9(10), 2014 [401-406]

Effect of Blueberry (*Vaccinium myrtillus)* on growth and survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* for production of probiotic milk and yoghurt

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ABSTRACT

This study was undertaken to determine the suitability of different doses (0, 0.1, 0.2 and 0.3%) of blueberry (*Vaccinium myrtillus)* on viability and growth of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in milk and yoghurt during 21 day refrigerated storage for production of probiotic milk and yoghurt. In order to determine the effects of different doses of blueberry on the growth of two probiotic bacteria, *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in milk and yoghurt produced, firs t 0.33 gram (LAFT_L10)*Lactobacillus acidophilus* was added to 1 liter low-fat sterilized milk, separately.

Then the samples were incubated at 38°C then the products were put in the refrigerator. Changes in pH, acidity and viable cell counts during incubation and permanence period were measured. In all samples that contained *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, it was observed that the increased Blueberry (*Vaccinium myrtillus)* concentration has effect on increasing acidity, decreasing pH, good color and taste and microbial count. The shelf lives of products were determined for 21 days. All the results suggest that Blueberry (*Vaccinium myrtillus)* promoted the metabolism of lactic acid bacteria in milk and yoghurt.

KEYWORDS

Blueberry;
Probiotic;
Lactobacillus acidophilus.
Bifidobacterium bifidum.

INTRODUCTION

Probiotic microbes are live organisms that are used by eating and condition application in repellent numberand cause creation of one or many healthy effects on host body. The ability of probiotics to withstand the normal acidic conditions of the gastric juices and the other microorganisms, allow them to be established in the intestinal tract[1]

Recently, the food biotechnology industry has developed a number of commercial products containing a single probiotic strain or bacterial associations of vari-

ous complexities.

Also, Lactic Acid Bacteria (LAB) and its metabolites have shown to play an important role in improving microbiological quality and shelf-life of many fermented food products. Dairy products have long been consumed by consumers and provide a good example of bio-preservation[2].

Today LAB is a focus of intensive international research for its pivotal role in most fermented foods.

Furthermore, LAB strains synthesize short chain fatty acids, vitamins, and exopolysaccharides (EPS) that are employed in the manufacturing of fermented milk to improve its texture and viscosity[3].

Recently design and product of probiotic

productions with basic plant are considered for both

rule of health nature of these food material groups

(protein, fiber, vitamin, solute) and variation creation in

product and consume[4].

traditional medicinal plants such as Blueberry (*Vaccinium myrtillus)* has been proved to provide important therapeutic values. The plant of Blueberry (*Vaccinium myrtillus),* with the common Persian names of "Ghareghat, is found natively in Europe, northern Asia, Greenland, Western Canada, and the Western United States.and it is found in north and north west parts of Iran.

Vaccinium myrtillus has been used for nearly 1,000 years in traditional European medicine. *Vaccinium myrtillus* fruits have been used in the traditional Austrian medicine internally (directly or as tea or liqueur) for treatment of disorders of the gastrointestinal tract and diabetes.

In Iranian traditional medicine, Bilberry leaf and fruit has been used for different conditions, including diabetes or high blood pressure.

The limited researches about therapeutic property of this plant are reported antibacterial and antifungal activity of this plant. Blueberry (*Vaccinium myrtillus)* is also very strong Antioxidant.

In the present study, we investigate first, the ability of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* to grow and survive in presence of Blueberry (*Vaccinium myrtillus)* during 21 day of refrigerated storage and evaluate the organoleptic properties of milk and yoghurt

MATERIALS AND METHODS

Materials

These included Blueberry (*Vaccinium myrtillus)* low-fat milk and yoghurt from supermarket, lyophilized *Lactobacillus acidophilus(LAFT_10)* and *Bifidobacterium bifidum* (CHR Hansen Company, Denmar),and MRS Agar and MRS Broth (Merck Company, Germany).

METHODS

Preparation of probiotic *Bifidobacterium Bifidum* milk containing Blueberry (*Vaccinium myrtillus)* powder

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. 0.33 gram of lyophilized of The starter (*Bifidobacterium bifidum*) was added directly to all the containers, followed by adding Blueberry powder of 0 (Control sample), 0.1, 0.2, and 0.3% to all the containers, respectively and finally they were placed in the incubator at 38°C. The acidity test was performed approximately every 2 hours until reaching 42° Dornic. The samples were then taken out of incubator and transferred to a refrigerator and stored at 2°C. The produced probiotic milk was evaluated once every 7 days by counting the microbes using direct counting method[9-11].

Preparation of probiotic *Bifidobacterium bifidum* yoghurt containing Blueberry (*Vaccinium myrtillus) powder* at the second passage

To produce *Biidobacterium bifidum* yoghurt in this stage, after providing 4 containers, 1 liter of the low - fat sterilized probiotic milk (1.5 % fat) from the control group at first passage and the (1.5%) starter of low-fat yoghurt (1.5%) were added to each container. Different concentrations of Blueberry powder (0, 0.1, 0.2, and 0.3%) were added respectively to the containers and mixed properly so that Blueberry powder 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 and transferred to a refrigerator and stored at 2°C. The produced probiotic Blueberry yoghurt was evaluated every 7 days by counting the microbes using direct counting method

Preparation of probiotic *Lactobacillus acidophilus* milk containing containing Blueberry (*Vaccinium myrtillus)* powder at first passage

All the same procedures were followed as mentioned above with the difference of using *Lactobacillus acidophilus* instead of *Bifidobacterium bifidum.*

Preparation of probiotic *Lactobacillus acidophilus* yoghurt containing containing Blueberry (*Vaccinium myrtillus)* powder at second passage

All the same procedures were followed as mentioned above with the difference of using *Lactobacillus acidophilus* instead of *Bifidobacterium bifidum.*

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

RESULT

TABLE 1 and TABLE 2 show the acidity degrees of Blueberry milk and yoghurt in *Lactobacillus acidophilus* and *Bifidobacterium bifidum* samples during storage time in the refrigerator. The results of these tables show the positive correlation between increased `acidity value` and increased Blueberry concentration which the samples containing 0.3% Blueberry powder in milk and yoghurt had high acidity value than the other sample.

TABLE 3 and TABLE 4 show the growth rates of microbes in Blueberry milk and yoghurt in *Lactobacillus acidophilus* and *Bifidobacterium bifidum* samples at storage time. The results show that the growth rate of bacteria was increased by increasing the concentration of Blueberry powder and reached the desired acidity at shorter period.

TABLE 6 and 7 shows the microbial growth on MRS-A cultivation environment of *Lactobacillus acidophilus* Blueberry milk and yoghurt at refrigerator during 21 day of storage. The samples containing 0.3% Blueberry powder possessed the highest count of bacteria.

TABLE 1 : The acidity level based on Dornic degree in the Blueberry *Lactobacillus acidophilus and Bifidobacterium bifidum* milk within 21-day storage in the refrigerator

Blueberry Lacto	2 day	7 day	14 day	21 day	Blueberry Bifido	2 day	7 day	14 day	21 day
%0	41°D	44°D	49°D	45°D	%0	42°D	45°D	51°D	47°D
%0/1	43°D	46°D	52°D	48°D	%0/1	44°D	48°D	54°D	51°D
%0/2	44°D	49°D	55°D	50°D	%0/2	45°D	51°D	60°D	58°D
%0/3	45°D	50°D	65°D	58°D	%0/3	46°D	53°D	68°D	64°D

TABLE 2 : The acidity level based on Dornic degree in the Blueberry *Lactobacillus acidophilus and Bifidobacterium bifidum* yoghurt within 21-day storage in the refrigerator

Blueberry Lacto	2 day	7 day	14 day	21 day	Blueberry Bifido	2 day	7 day	14 day	21 day
%0	71°D	80°D	85°D	74°D	%0	71°D	78°D	80°D	73°D
%0/1	75°D	90°D	96°D	80°D	%0/1	76°D	81°D	91°D	77°D
%0/2	82°D	93°D	100°D	85°D	%0/2	78°D	83°D	98°D	80°D
%0/3	90°D	95°D	106°D	92°D	%0/3	80°D	87°D	101°D	83°D

TABLE 3 : Total bacterial count of *Lactobacillus acidophilus* and Bifidobacterium in the Blueberry milk within 21-day

Blueberry Lacto	2 day	7 day	14 day	21 day	Blueberry Bifido	2 day	7 day	14 day	21 day
%0	35×10^{9}	45×10^{9}	5×10^{10}	475×10^{8}	%0	4×10^{10}	5×10^{10}	65×10^{9}	6×10^{10}
%0/1	475×10^{8}	575×10^{8}	65×10^{9}	6×10^{10}	%0/1	575×10^{8}	65×10^{9}	825×10^{8}	75×10^{9}
%0/2	55×10^{9}	625×10^{8}	725×10^{8}	675×10^{8}	%0/2	625×10^{8}	75×10^{9}	925×10^{8}	825×10^{8}
%0/3	625×10^{8}	7×10^{10}	825×10^{8}	775×10^{8}	%0/3	7×10^{10}	825×10^{8}	10×10^{10}	95×10^{9}

FULL PAPER

TABLE 4 : Total bacterial count of *Lactobacillus acidophilus and Bifidobacterium bifidum* in the Blueberry yoghurt within 21-day

Blueberry Lacto	2 day	7 day	14 day	21 day	Blueberry Bifido	2 day	7 day	14 day	21 day
%0	5×10^{10}	575×10^{8}	75×10^{9}	525×10^{8}	%0	425×10^{8}	475×10^{8}	7×10^{10}	45×10^{9}
%0/1	625×10^{8}	675×10^{8}	9×10^{10}	65×10^{9}	%0/1	525×10^{8}	675×10^{8}	825×10^{8}	6×10^{10}
%0/2	7×10^{10}	775×10^{8}	1020×10^{9}	725×10^{8}	%0/2	6×10^{10}	75×10^{9}	95×10^{9}	65×10^{9}
%0/3	75×10^{9}	875×10^{8}	1120×10^{9}	775×10^{8}	%0/3	65×10^{9}	8×10^{10}	1070×10^{8}	7×10^{10}

TABLE 5 : The acidity level based on Dornic degree in the Blueberry *Lactobacillus acidophilus* and *Bifidobacterium bifidum* yoghurt in productiom day

h Lacto	0 h	2 h	4 h	6 h	8 h	h Bifido	0 h	2 h	4 h	6 h	8 h
%0	20°D	21°D	40°D	59°D	72°D	%0	20°D	22°D	39°D	64°D	72°D
%0/1	21°D	28°D	57°D	70°D		%0/1	21°D	27°D	55°D	68°D	72°D
%0/2	21°D	29°D	60°D	72°D		%0/2	22°D	29°D	56°D	70°D	
%0/3	22°D	30°D	62°D	72°D		%0/3	22°D	30°D	58°D	71°D	

The microbial growth on MRS-A cultivation environment of *Bifidobacterium bifidum* Blueberry milk and yoghurt at refrigerator during 21 days was poor because *Bifidobacterium bifidum* has good growth on MRS Broth. The microbial growth of *Bifidobacterium bifidum* on MRS Broth was high. It was observed that *Bifidobacterium bifidum* has high inhibitory activity in MRS Agar during 21 days of storage. These results showed that Blueberry was suitable for this intestinal bacterium that was kept viable up to the end of fermentation (21days). All tested *Bifidobacterium bifidum* was capable of growing well on Blueberry milk and yoghurt without nutrient supplementation.

TABLE 6: The microbial growth on MRS-A cultivation environment of *Lactobacillus acidophilus* in Blueberry milk at refrigerator during21 days insolubility

Dill Lacto	2 day	7 day	14 day	21 day
%0	275×10^{9}	325×10^{9}	425×10^{9}	375×10^{9}
%0/1	315×10^{9}	40×10^{10}	60×10^{10}	525×10^{9}
%0/2	375×10^{9}	47×10^{10}	95×10^{10}	813×10^{9}
%0/3	40×10^{10}	50×10^{10}	133×10^{10}	120×10^{10}

TABLE 7 : The microbial growth on Broth cultivation environment of *Bifidobacterium bifidum* in Blueberry milk at refrigerator during21 days insolubility

	10^{-2}				10^{-3}				10^{-4}				10^{-5}				10^{-6}			
day	2	7	14	21	2	7	14	21	2	7	14	21	2	7	14	21	2	7	14	21
0%	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
%0/1	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-
%0/2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-
% 0/3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE 8 : The microbial growth on MRS-A cultivation environment of *Lactobacillus acidophilus* in Blueberry yoghurt at refrigerator during21 days insolubility

Blueberry Lacto	2 day	7 day	14 day	21 day
%0	30×10^{10}	325×10^{9}	65×10^{10}	50×10^{10}
%0/1	38×10^{10}	45×10^{10}	875×10^{9}	675×10^{9}
%0/2	625×10^{9}	775×10^{9}	117×10^{10}	725×10^{9}
%0/3	100×10^{10}	145×10^{10}	1725×10^{9}	1125×10^{9}

DISCUSSION

In the present study, the effects of Blueberry (*Vaccinium myrtillus)* on the growth and viability of the bacteria *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in probiotic milk and yoghurt were investigated. The acidity, pH and survival of the bacteria in Blueberry probiotic milk and yoghurt were evaluated at 2 hours intervals till reaching 42°Dornic acidity degrees for milk and 90°Dornic degree for yoghurt in the incubator at 38°C. At the first hours of production, the *Lactobacillus acidophilus* milk containing 0.3% and 0.2 Blueberry powder reached the acidity of

TABLE 9 : The microbial growth on Broth cultivation environment of *Bifidobacterium bifidum* in Blueberry yoghurt at refrigerator during21 days insolubility

	10^{-2}				10^{-3}				10^{-4}				10^{-5}				10^{-6}			
day	2	7	14	21	2	7	14	21	2	7	14	21	2	7	14	21	2	7	14	21
0%	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
%0/1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
%0/2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
% 0/3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

42°Dornic earliest, followed by 0.5, 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.In direct microbial counting in first day, the highest counts were sequentially in the samples with 0.10.2, & 0.3% and the controls, indicating the positive correlation between increased bacterial growth and increased Blueberry concentration. Upon evaluation of the cultured samples on MRS agar media, the same correlation was revealed. The *Lactobacillus acidophilus* yoghurt with 0.3% Blueberry powder reached the acidity of 90°Dornic earliest, followed by the samples with 0.2, & 0.1% and the control, Once they reached this acidity level, they were transferred to a refrigerator at 2°C. The storage time in the refrigerator was found to be 21days.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.

The sensory evaluation was performed by 50 participants for the probiotic *Lactobacillus acidophilus* yoghurt with varying concentrations of Blueberry after seven days. There were significant differences between the samples and it was shown that the 0.1% of Blueberry has the best favorable taste, color, scent and thickness.

In direct microbial counting in the first day, the highest counts were sequentially in the samples with 0.2% and 0.3 % Blueberry and the results indicated positive correlation between increased bacterial growth and increased Blueberry concentration. Upon evaluation of the cultured samples on MRS Agar media, the same correlation was revealed[7]. During the storage time in the refrigerator which was 21 days the acidity level of the control sample with *Lactobacillus acidophilus and Bifidobacterium bifidum* were lower than other andacidity level of 0.3 % Blueberry with *Lactobacillusacidophilus and Bifidobacterium bifidum* were higher than other. The minimum required level of probiotic bacteria to be useful for the consum *Bifidobacterium bifidum*, in probiotic products, it was demonstrated that the shelf life for the acidity reaching the desired level during incubation decreases 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.

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 yoghurt with only

Lactobacillusacidophilus tasted sourer than the yoghurt with both bacteria. The products containing *Bifidobacteriumbifidum*, compared to those with *Lactobacillusacidophilus*, 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[13].

In another study addressing the effect of cinnamon on the bacterial growth, it was demonstrated that the increased cinnamon concentration promoted the growth of the bacteria in probiotic milk and yoghurt[14].

The results of the experiments in this work showed that Blueberry was a suitable support for these intestinal bacteria that were kept viable up to the end of fermentation (21days). All tested *Bifidobacterium bifidum* and *Lactobacillus acidophilus* were capable of growing

well on Blueberry milk and yoghurt without nutrient supplementation.*acidophilus* had the highest viability in all of the products investigated.but the taste of *Bifidobacterium bifidum* was better.

The survival of probiotic bacteria in refrigerated conditions for at least 21 days were in number of greater than 109cfu. mLG1 which is essential if a product should have probiotic properties. It is important to emphasize that all the products possessed excellent stability during 21 days of storage. The sensory scores of the products were high and foregoing results it can be concluded that Blueberry can be successfully used in formulation of dairy products.

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