The survey of survival of probiotic Bifidobacterium bifidum and its effect on microbial and physiochemical of fruit drinking yogurt

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Abstract: Probiotics are the microorganisms that by improving the microbial balance of microflora in the body can exert useful benefits. Thus, applying the probiotic bacteria in production of yogurt leads into the production of probiotic yogurt. In the current study, besides producing probiotic product, the survival of the bacterium and its effect on microbial, physiochemical and sensory properties of fruit drinking yogurt was investigated. At first from raw milk 1.5%, yogurt was produced and then fruit drinking yogurt and then probiotic bacteria 0.1, 0.01 dilutions were added to the product and after packing stages was cooled at 4 for 21 days. The results showed that acidity reduction and pH trend in the product with dilution 0.1 is less than the product with dilution 0.01 and control sample. The survival of the bacterium during 21 days is acceptable for probiotic product.
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1. Introduction

The history of the discovery, production and using fermentation productions of milk dates back to 10 thousand years BC in Middle East including Balkan and among Ariayi tribes located in Iran and Turkey. The Russian bacteriologist Eli Metchnikoff used bacillus as endospore bacteria in modern yogurt making. Probiotics are a kind of supplement consisting of potential bacteria and fungus. The difference of probiotic with other microorganisms is the useful effects for the host and healthy effects of probiotics via their biological activities inside the body after being located in various parts (1). It should be said that to apply the highest useful effects by these bacteria, some conditions as the selection of the type of probiotic bacterium and its application and controlling the underlying conditions on product production stages and other cases are necessary and one of the important issues is the survival of probiotic bacteria in the product to keep the survival of these bacteria during the storage. As probiotic bacteria can have the considerable positive effects (2, 3). Lactic acid producing bacteria are seen in various parts of the body. The importance of microflora in the intestine of a normal person as the inhibiting factor against pathogen microorganisms was defined in 19th century (4). Lactic acid bacteria are the microflora members of the boy and these bacteria can be traditionally used in the production of fermented dairy products (5, 6). In reduction of cholesterol, anti-microbial activity against the pathogens, good balance in natural microflora and reduction of lactose intolerance and most of the performances are important (3-5). As cholesterol is of great importance in some attributes as making plasma membrane, steroid hormones, bile salts and fetus evolution and its increase is harmful and it creates solid crystals in the cells leading into the cell death and its increase in the blood leads into atherosclerosis and cardio-vascular diseases and its increase in bile cause bile stone (6). Therefore, using these microorganisms in producing the drinking yogurt can create a high quality and valuable product. It is hoped that by producing such products, we can made a positive change for individual's health.

2. Materials and methods

Starter including Lactobacillus acidophilus and Lactobacillus thermophilus (dried frozen), DVS was obtained from Chr. Hansen's company

Milk 1.5% fat from Pegah factory

Probiotic Bifidobacterium bifidum as dried frozen, DVS from Chr. Hansen's company

Sugar, pectin and fruit extract

In this study, two commercial bacteria culture including mixed culture of yogurt as Lactobacillus del- brueckii subsp. bulgaricus and Streptococcus thermophilus (yoflex mild 1.0 k.h) and single culture of probiotic Bifidobacterium bifidum as frozen and dried, DVS from Chr. Hansen's company were applied. To activate the combinational culture of yogurt, the homogenized milk of yogurt production of Tehran pasteurized milk (Pegah) was used and after thermal process 95 for 15 min, a pack of 50 unit of starter was added as sterile and according to the

instruction to one liter of it and was dissolved at 20 for 15 min and was used as volume percent in inoculation time. Probiotic single cultivation (25-gr package) was used as weight percent in septic conditions. To produce the basic milk, the milk of Pegah factory was used.

Then, thermal process was applied at 95 for 15 min and after the reduction of temperature to good temperature (37), inoculation was done by yogurt starter culture (0.2% volume-volume). Immediately after the completion of the time, the yogurt with pH 4.50 was cooled to 4 in cold water. After adding water (15% volume-volume) and sugar (7 weight-volume) and 0.3% pectin stabilizer and 3% fruit concentrate were added and the product was mixed and diluted and was pasteurized at 85 for 5 min and was homogenized at 150 bar and then it was cooled to 4 and probiotic bacterium at 0.1, 0.01 dilution was added. In 250cc bottles, PET was packed and finally the product was kept at 4 for 21 days. The control sample was produced without adding bifidobacterium bifidum and only via milk fermentation by yogurt cultivation and other stages.

During the storage time, microbial analyses including (the count of probiotic bacteria, mould and coliform count), physicochemical including (pH, acidity and density) were done.

The acidity was measured by Durnic unit and each durnic degree showed the presence of 1/100% lactic acid of the sample. Determining the acidity was done based on Iran national standard No. 2582.

pH showed the logarithm of hydrogen ion concentration in the sample. pH was determined by Iran national standard No. 2582.

To produce the diluents solution, two ringer pills reached 1 lit volume with distilled water and Ringer's solution was prepared. Then, we pour inside the clean tubes, 9mL of Ringer's solution and close it with cotton and do the autoclave.

3. Results

Table 1: The number of probiotic bacterial in dilution 0.1, 0.01 during the storage

Microbial tests	Day 0		Day 7		Day 14		Day 21	
Total number of forms	0		0		0		0	
The number of fermentation mould	0		0		0		0	
The dilution of probiotic bacteria	0.01	0.1	0.01	0.1	0.01	0.1	0.01	0.1
The number of probiotic bacteria	10^6	10^8	10^6	10^8	10^6	10^8	10^6	10^8
	81	85	69	78	47	62	27	7

Table 2: physicochemical tests of dilution (0.1)

Physicochemical tests	Day 0	Day 7	Day 14	Day 21
Acidity	70	72	75	85
pH	4.28	4.24	4.18	4.04
Density	52	52	52	54

Table 3: physicochemical tests of dilution (0.01)

Physicochemical tests	Day 0	Day 7	Day 14	Day 21
Acidity	70	73	77	93
pH	4.28	4.22	4.15	3.97
Density	52	52	52	54

Table 4: physicochemical tests of control sample

Physicochemical tests	Day 0	Day 7	Day 14	Day 21
Acidity	70	73	78	104
pH	4.28	4.19	4.13	3.85
Density	52	52	52	54

4. Discussion and conclusion

Now, yogurt is the highly applied probiotic product in the world. The reason is unique sensory attributes of this product and its healthy value (7). Drinking Yogurt with optimized attributes from biology viability, sensory attributes and economical value can be of great importance. Probiotic drinking yogurt is physically similar to the normal drinking yogurt but each mL of it has 10 live probiotic bacteria. From nutrient view, its consumption responds the needs of amine acid and the main fatty acids of the body. In addition, probiotic bacteria activity increases the access of body biologic to the ions by which calcium, iron, magnesium, copper, sodium, etc are absorbed better (7, 8).

The changes of acidity and pH during the survival time were registered in the fridge. The results of registering the change of these two factors in all the samples showed that the samples with high probiotic bacterium dose, it had less acidity and pH and it can be evaluated as due to the effect of probiotic bacteria activity. The results showed that the increase of acidity and reduction of pH during 14 to 21 days was done with more speed and it can be dedicated to the reduction of probiotic bacteria.

During the survival, probiotic bacteria didn't increase and this is due to adding bacteria at the end of product production process. The decrease of bacteria is high during the storage. This is due to the high sensitivity of it to oxygen, high acidity and low pH, the need to growth supplements, small organic azoth and vitamins and low potential (7).

The movement of bacteria under the microscope showed the live bacteria after 21 days of survival and

the high dose product had the maximum activity. The major problem of production of probiotic drinking yogurt is losing viability or probiotics survival in production and storage to normal drinking yogurt.

The minimum amount of bacteria in probiotic products is 106 and in this study the bacteria were 108,106 microbes. Thus, the probiotic microbes' properties are created in the consumer.

The term “probiotic” was recognized as growth factors and was introduced for the first time in 1965 by Lilly and Stillwell. Despite the antibiotics, probiotics as microbial derivative factors stimulated the growth of other microorganisms (1).

In 2005, Daniel Kamane investigated the anti-cancer properties of probiotics and considered them with initial useful properties for the host to avoid intestine infection and other useful effects including the reduction of allergy, controlling cholesterol, combining body immunity and prevention of colon cancer (9).

Kailasapathy and Rybka in 1977 proposed that probiotic should be added before and at the same time with the starter and if it is added after the fermentation, there is not growth (10).

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