World Applied Sciences Journal 18 (10): 1389-1393, 2012
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2012.18.10.63242

Effect of Permeate on Growth and Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* for Production of Probiotic Nutritive Beverages

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Abstract: Permeate is the by- product of the treatment of milk by using ultra filtration process. Permeate retains about 80% of the initial lactose of the treated milk that could cause a high BOD so it could be, a factor of environmental concern. This study was undertaken to determine the suitability of permeate for production of probiotic beverage by two lactic acid bacteria (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) that can be used in many formulation in food technology. In this Investigation, four *Lactobacillus acidophilus* and four *Bifidobacterium bifidum* permeate beverages were prepared containing permeate with concentrations of 0 (control), 5, 10 and 15%. Then the samples were incubated at 38°C for 6 hours and 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 permeate concentration has effect on increasing acidity, decreasing pH, good color and taste and microbial count. The shelf life of the products was evaluated after 1, 7, 14 and 21 days. After 21days of cool storage the bacterial count was higher than 10^8cfu.ml^{-1}. The results showed that permeate was a suitable support for these Intestinal bacteria. *Lactobacillus acidophilus* had the highest viability in all of the products investigated. In day seven, the products were sensory evaluated. The higher sensory scores were attained by beverage with *lactobacillus acidophilus*: 10%permeate and 20gram mixed date received the best scores after 7days of storage, but these scores decreased as storage time elapsed.

Key words: Permeate · Probiotic · *Lactobacillus acidophilus* · *Bifidobacterium bifidum*

INTRODUCTION

Permeate is an important by-product of the treatment of milk by using ultra filtration process in cheese industry. Permeate contains lactose as the major constituent in addition to water soluble vitamins and the salts of milk. Therefore, permeate can be considered as a solution of nutritious significance. Permeate retains about 80% of the initial lactose of the treated milk. The milk permeate is practically free of N-compounds and thus unsuitable for animal feeding [1].

Consequently, it is a subject of environmental concern due to its high biological oxygen demand (BOD). Thus, the question of milk permeate disposal/utilization is effectively a question of lactose disposal/utilization: utilizing lactose economically is crucial to the treatment of this high BOD wastewater, specially for small factories. Traditional methods of whey and permeates utilization include direct animal feeding by nearby farmers, direct soil application and sewage disposal [2-4].

Today, because of growing concern about environmental pollution, sewage disposal is undesirable due to the high BOD. Some investigation has focused on conversion of whey and permeates to forms that are suitable for human consumption [5].

To enrich permeate solution therefore increase its nutritional value, probiotic microorganisms are one of the best choices for production of a fermented beverage based on permeate.

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Probiotics by definition are "live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance". Probiotic bacteria like bifidobacteria and lactobacilli are natural inhabitants of the human gut. They affect human health by improving the gut microbiota balance beneficially and the defenses against pathogens [6- 10].

This study was undertaken to determine the suitability of permeate for production of probiotic beverages by two lactic acid bacteria (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) that can be used in many formulation in food technology.

Considering its potential, the purpose of this study was to define the growth and survival of probiotic bacteria in permeate solution for possible production of a nutritive highly valuable permeate beverage.

MATERIALS AND METHODS

Sample Collection and Preparation: Permeate powder was obtained from Shiraz Ramak Dairy Company (Shiraz, Iran). It was a byproduct from the UF cow's skim milk. It was prepared at 50°C using spiral-wound module membrane. The permeate was immediately heated in a water bath at 80°C for 10 min and cooled to 4°C and kept frozen at -20°C until use. Date as a natural sugar was locally purchased (Kazerun, Iran). Lyophilized Probiotic strains, *Lactobacillus acidophilus* La-5 and *Bifidobacterium bifidum* Bb-12 were obtained from CHR Hansen Company (CHR-Hansen, Denmark). MRS Agar culture medium was used for carrying out the microbial test (MERCK, Germany).

Methods: In order to produce the beverages based on permeate that contained probiotic bacterium, first eight containers each containing 1 liter sterile water were considered as our eight groups. Twenty gram mixed date as natural sugar was added directly to all the containers, after that permeate powder 0% (control sample), 5% (50gram), 10% (100gram) and 15% (150gram) were separately added to all the containers and they were pasteurized.

Effect of Permeate on the Production of Probiotic *Lactobacillus Acidophilus* Beverages: After pasteurization, to determine the effect of different doses of permeate (0, 5, 10 and 15%) on increasing the growth of probiotic bacteria; *Lactobacillus acidophilus*, 0.33 gram of lyophilized *Lactobacillus acidophilus*, was added to four containers containing pasteurized permeate solutions. Then the samples were incubated at 38°C for 6 hours. The acidity and pH were measured every 2 hours during the incubation period. After 6 hours all samples were taken out of incubation, transferred to a refrigerator and stored at 2°C. The shelf life of the products was evaluated after 1, 7, 14 and 21 days [11-13].

Effect of Permeate on Production of Probiotic *Bifidobacterium Bifidum* Beverages: After pasteurization, to determine the effect of different doses of permeate (0, 5, 10 and 15%) on the growth of *Bifidobacterium bifidum,* 0.33 gram of lyophilized *Bifidobacterium bifidum*, was added to four containers containing pasteurized permeate solutions and all the containers were placed in the incubator at 38°C for 6 hours. the acidity test was done every 2 hours during incubation period. after 6 hours the products were put in the refrigerator at 2°C. The shelf lives of the products were evaluated after 1, 7, 14 and 21 days [11-13].

Sensory Evaluations of the Probiotic Permeate Beverages: In day seven, the products were sensory evaluated. The sensory scores of the beverages were high and acceptance. The higher sensory scores were attained by beverage with *lactobacillus acidophilus*: 10%permeate and 20gram mixed date received the best scores after 7days of storage, but these scores decreased as storage time elapsed.

Statistical Analysis: All the above experiments were repeated three times. SPSS17 was used for one-way analysis of variance for all data and significant differences ($p < 0.05$) among means were determined by the least significant difference test.

RESULTS

Table 1 shows the acidity in Dornic degree in *Lactobacillus acidophilus* permeate beverages at refrigerator during 21 days. Table 2 shows microbial growth by direct count of *Lactobacillus acidophilus* permeate beverage stored at refrigerator during 21 days. Table 3 shows the growth on MRS-A cultivation environment of *Lactobacillus acidophilus* permeate beverage at refrigerator during 21 days storage. Table 4 shows the acidity in Dornic degree in *Bifidobacterium bifidum* permeate beverages at refrigerator during 21 days. Table 5 shows microbial growth by direct count of *Bifidobacterium bifidum* permeate beverage at refrigerator during 21 days.

Table 1: The acidity level based on Dornic degree in the Lactobacillus acidophilus permeate beverage within 21 days of storage in the refrigerator.

	Acidity level in Dornic degree			
Permeatebeverage	1 day	7day	14day	21 day
0%	5	4	4	5
5%	22	24	23	20
10%	34	36	54	44
15%	46	69	69	59

Table 2: Total bacterial count of the Lactobacillus acidophilus permeate beverage

Permeate beverage	1 day	7day	14 day	21day
0%	$10^8\times175$	$10^9\times15$	$10^9\times15$	$10^{10}\times1$
5%	$10^9\times35$	$10^8\times225$	$10^8\times175$	$10^9\times15$
10%	$10^{10}\times4$	$10^8\times175$	$10^9\times15$	$10^8\times125$
15%	$10^{10}\times5$	$10^{10}\times3$	$10^8\times225$	$10^{10}\times2$

Table 3: The microbial growth on MRS-A cultivation environment of Lactobacillus acidophilus permeate beverage at refrigerator during 21 days insolubility

Permeate beverage	1 day	7 day	14 day	21 day
0%	$10^{10}\times3$	$10^9\times25$	0	0
5%	$10^9\times625$	$10^9\times575$	75×10^8	$10^{10}\times2$
10%	$10^9\times187$	$10^{10}\times75$	1010×3	$10^9\times147$
15%	$10^{10}\times33$	$10^{10}\times75$	147×10^9	$10^9\times55$

Table 4: The acidity level based on Dornic degree in the Bifidobacterium bifidum permeate beverage within 21-day storage in the refrigerator

	Acidity level in Dornic degree			
Permeate beverage	1 day	7 day	14 day	21 day
0%	11	10	14	14
5%	32	31	27	25
10%	42	53	50	36
15%	59	54	67	42

Table 5: Total bacterial count of the Bifidobacterium bifidum permeate beverage

Permeate beverage	1 day	7 day	14 day	21 day
0%	$10^9\times15$	$10^8\times175$	$10^8\times175$	$10^8\times125$
5%	$10^{10}\times3$	$10^8\times175$	$10^{10}\times2$	$10^9\times15$
10%	$10^8\times525$	$10^8\times375$	$10^8\times225$	$10^9\times15$
15%	$10^{10}\times5$	$10^{10}\times4$	$10^{10}\times3$	$10^8\times225$

The microbial growth on MRS-A cultivation environment of *Bifidobacterium bifidum* permeate beverages 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. This results showed that permeate was suitable for this Intestinal bacteria that was kept viable up to the end of fermentation (21days). All tested *Bifidobacterium bifidum* was capable of growing well on permeate beverages without nutrient supplementation.

DISCUSSION

In recent years, probiotic bacteria, as the food additives, have been introduced into numerous foods, of which the dairy products have played an important role in carrying these bacteria (such as *Bifidobacterium bifidum* and *Lactobacillus acidophilus*). Eating regularly the sufficient amounts of living cells "the minimum treatment" is required if the consumer is to benefit from the probiotic products [14].

An important factor that affects the survival of probiotic bacterial strains in food is pH; survival is constrained at low pH values. Hence, permeate should be a good food carrier for probiotic strains because it has a pH in the range 4.7-5.8 and never lower than 4.5 even after 21 days of storage.

Following some initial experimentations, we came to the conclusion that the use of high concentration (15%) of permeate powder could enhance the growth of the bacterium *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. Similarly, the addition of date as sugar to the products promoted the bacterial growth.

According to table 1 the increased permeate concentration resulted in acidity so permeate concentration has a direct influence on growth of bacteria. In control sample the growth of bacteria was very slow and it can be concluded that permeate powder has an important role for growth and survival of probiotic bacteria.

In direct microbial counting in the first day, the highest counts were sequentially in the samples with 10% and 15% permeate and the results indicated positive correlation between increased bacterial growth and increased permeate concentration.

Upon evaluation of the cultured samples on MRS Agar media, the same correlation was revealed. During the storage time in the refrigerator which was 21 days the acidity level of the control sample with *Lactobacillus acidophilus* was lower than other samples.

The minimum required level of probiotic bacteria to be useful for the consumer's body is 10^7 CFU.ml^{-1} of living bacteria and the level in the present study was found to be 10^{10}, thus, it could be beneficial for the consumers.

Upon evaluation of the samples on MRS Agar, *Lactobacillus acidophilus* with permeate powder had the counts equal to logarithmic 10^9 until 21st day and the sample products with 10% and 15% permeate possessed the highest count of bacteria.

The sensory evaluation was performed for the probiotic *Lactobacillus acidophilus* permeate beverage, after seven days in term of scent, color and taste. The

higher sensory scores were attained by beverage with *lactobacillus acidophilus*: 10% permeate and 20gram mixed date received the best scores after 7days of storage, but these scores decreased as storage time elapsed.

The pH of the products with probiotic beverage containing either of the bacteria during the incubation and shelf life was not lower than 4.5. As for the probiotic permeate beverage, the respective pH reached 5.03 during production and it came to 4.96 during shelf life in the beverage with *Bifidobacterium bifidum*.

Bifidobacterium bifidum beverage with 15% permeate had highest acidity and we came to the conclusion that the use of high concentration (15%) of permeate powder could enhance the growth of the bacterium *Bifidobacterium bifidum*. Similarly, the addition of date as sugar to the products promoted the bacterial growth.

The permanence of the product in the refrigerator was determined 21 days during which the acidity of the control sample was lower than other samples.

The bactericidal and inhibitory effect of low pH was stronger for *Bifidobacterium bifidum* than that for *Lactobacillus acidophilus* and it seems that during the storage time and enhanced fermentation process, lowering of pH caused decreased growth of *Bifidobacterium bifidum*.

No significant difference was observed in the *Bifidobacterium bifidum* permeate beverage in terms of color, taste and scent.

The sample with 15% had highest bacterial counts, as revealed in the evaluation of the samples on MRS agar medium. The increased permeate concentration in *Lactobacillus acidoph*ilus and *Bifidobacterium bifidum* permeate beverage led to bacterial growth and faster favorable acidity.

Comparing with the reported studies on malt, permeate and malt extract; as for malt extract, the increased malt concentration enhanced acidity and had shorter shelf life but permeate beverage had a longer shelf life than malt extract [11].

As for the products with soya, the increased concentration led to enhanced acidity, as we observed in permeate beverage. The increased concentration of malt and soya caused increase in the microorganism growth and rising acidity level which in turn resulted in shorter incubation time for the desired acidity [11, 12] .

In a study on the effects of soya powder on the growth of the bacteria, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, in probiotic products, it was demonstrated that the shelf life for the acidity reaching the desired level during incubation decreased 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 decreased [12].

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 the yoghurt with only *Lactobacillus acidophilus* tasted sourer than the yoghurt with both bacteria. The products containing *Bifidobacterium bifidum*, compared to those with *Lactobacillus acidophilus*, 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 spearmint on the bacterial growth, it was demonstrated that the increased spearmint concentration promoted the growth of the bacteria in probiotic milk and yoghurt [15].

In another study addressing the effect of juice on the bacterial growth, it was demonstrated that the increased juice product promoted the growth of the bacteria in probiotic orange and apple [16].

The results of the experiments in this paper showed that permeate was a suitable support for these Intestinal bacteria. *Lactobacillus acidophilus* had the highest viability in all of the products investigated.The survival of probiotic bacteria in refrigerated conditions for at least 21 days were in number of greater than 10^8 cfu•mL^{-1} which is essential if a product should have probiotic properties. It is important to emphasize that all the beverages produced possessed excellent stability during 21 days of storage. The sensory scores of the beverages were high and acceptance. From the foregoing results it can be concluded that permeate can be successfully used in the preparation of nutritive beverages. Addition of strawberry or mango may produce more acceptable probiotic permeate beverages.

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