ISSN : 0974 - 7435 Volume 9 Issue 1

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 9(1), 2014 [14-18]

Effect of gginger on the growth of *Lactobacillus casei* and *Lactobacillus paracasei* in probiotic milk

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ABSTRACT

The objective of this study was to investigate the effect of different doses of ginger on the growth of *Lactobacillus casei* and *Lactobacillus paracasei* (separated from Iranian dairy products) in milk produced at one step. The product was then examined in terms of pH, acidity and microbe counting during incubator setting period. In the milk samples with *Lactobacillus casei*, the control sample reached acidity level more quickly and in the milk samples with *Lactobacillus paracasei*, the sample containing %1 ginger reached acidity level earlier than other samples. In the milk samples with either *Lactobacillus casei* or *Lactobacillus paracasei*, it was observed that during refrigeration the control sample had the most duration. The bioability of probiotic bacteria was measured by direct counting method. Duration of the product permanence was determined within 21 days. Upon examination of the results, it was revealed that the increased concentration of ginger had a positive effect on the growth of the probiotic bacteria, *Lactobacillus casei* and *Lactobacillus paracasei* in probiotic milk.

KEYWORDS

Probiotic;
Lactobacillus casei;
Lactobacillus paracasei;
Ginger;
Milk.

INTRODUCTION

Probiotics are "Living micro-organisms, which Upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition"[3]. Foods containing such bacteria fall within the "functional Foods" category and these are described as "foods claimed to have a positive effect on health"[13]. Functional foods should contain at least 10^7cfu g^{-1} probiotic bacteria and should be consumed at levels higher than 100 g day^{-1} to have positives effects on health[10]. Probiotic-containing products have become primary choise for the consumer because of their health attributes. Therefore, the market for such products has rapidly grown[11]. Many different strains and species of lactobacilli and bifidobacteria have been used commercially as probiotcs. It is well known that the probiotic bacteria have health-promoting effects and antagonistic activity against food-borne disease

agents[12].

Recently, the design and production of plant-based probiotic products have received much attention chiefly due to their natural health benefits (protein, fiber, vitamin and salts) and also because of the variety in their production. Therefore, it seems that the issue of producing probiotic foods with appropriate qualities will be a major research topic for prospective researchers[1].

Although, in the past decades, the synthetic chemical drugs that make use of separation mechanisms have been much in demand, their corresponding side effects are being gradually observed so much so that their irregular and improper consumption has turned out to be a critical issue.

On the contrary, the benefits of medicinal plants and their little or zero side effects have made them a proper substitute, highly appreciated by physicians and patients. Iran possesses a very rich source of such plants and herbs in the world in terms of variety and amount. On the contrary, the benefits of medicinal plants and their little or zero side effects have made them a proper substitute, highly appreciated by physicians and patients. Iran possesses a very rich source of such plants and herbs in the world in terms of variety and amount. The essence plants play a critical role in human life, and have been used for long by Iranians. Ginger is widely used around the world in foods as a spice. For centuries, it has been an important ingredient in Chinese, Ayurvedic and Tibb-Vnani herbal medicines for the treatment of catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes[2,5].

Currently, there is a renewed interest in ginger, and several scientific investigations aimed at isolation and identification of active constituents of ginger, scientific verification of its constituents, and verification of the basis of the use of ginger in some of several diseases and conditions[2].

The aim of the present study was to evaluate the effect of ginger on the growth of *Lactobacillus casei* and *Lactobacillus paracasei* in probiotic milk.

MATERIALS AND METHODS

Materials

Materials included dried ginger powder, Low fat sterilized milk from supermarket (%1/5 fat), lyophilize *Lactobacillus casei* (isolated from Iranian dairy products), lyophilize *Lactobacillus paracasei* (isolated from Iranian dairy products) and MRS Agar (Merk Company, Germany).

Effect of ginger on the production of probiotic *Lactobacillus casei* milk as first passage:

In order to produce the milk containing the probiotic bacterium *Lactobacillus casei*, Four containers each containing 250 cc of low-fat sterilized milk (%1.5 fat) were considered as our four groups. 0.1 gram starter (*Lactobacillus casei*) was added directly to all the containers, followed by adding ginger powder 0 (the control sample), 1, 2 and %3 to all the containers, respectively and finally they were placed in an incubator at 38 °C. The acidity test was performed approximately every 2 hours until reaching 84-87 °Dornic.

Effect of ginger on the production of probiotic *Lactobacillus paracasei* milk at the second passage:

To produce *Lactobacillus paracasei* milk, four containers each containing 1 liter of low-fat sterilized milk (%1.5 fat) were considered as our four groups. 0.2 gram starter (*Lactobacillus paracasei*) was added directly to all the containers, followed by adding ginger powder 0 (the control sample), 1, 2 and %3 to all the containers, respectively and finally they were placed in an incubator at 38 °C. The acidity test was performed approximately every 2 hours until reaching 89 °Dornic. After reaching *Lactobacillus casei* and *Lactobacillus paracasei* milk samples to 84-87 °Dornic and 89 °Dornic respectively, they were taken out of the incubator and mixed together and then transferred to a refrigerator and stored at 2 °C produced probiotic milk was evaluated once every 7 days by counting the microbes using direct counting method.

RESULTS

TABLE 1 shows the pH level in the ginger *L. casei* milk during incubation and TABLE 2 shows the acidity level in the ginger *L. casei* milk during the same time. TABLE 3 shows the pH level in the ginger *L. paracasei* milk during incubation, TABLE 4 shows the acidity

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degree in the ginger *L. paracasei* milk during incubation, TABLE 5 shows the PH level in the ginger *L. casei* and *L.paracasei* milk within 21-day storage in the refrigerator, TABLE 6 shows the acidity level base on Dornic degree in the ginger *L. casei* and *L.paracasei* milk within 21-day storage in the refrigerator and TABLE 7 shows the growth rate of microbes in the ginger *L. casei* and *L. paracasei* milk.

TABLE 1 : The pH level in the ginger *L. casei* milk during incubation

Ginger Milk (%)	00:00 (h)	03:30 (h)	05:00 (h)	06:00 (h)	08:30 (h)	11:00 (h)	12:00 (h)	15:30 (h)	16:30 (h)
0	↓	5/06	4/99	4/93	4/83	4/66	---	---	---
1	↓	5/62	5/54	5/44	5/27	5/14	5/03	4/9	4/93
2	↓	5/27	5/42	5/31	5/31	5/23	5/12	4/97	4/99
3	↓	5/79	5/71	5/73	5/64	5/70	5/67	5/60	5/58

TABLE 2 : The acidity level in the ginger *L. casei* milk during incubation

Ginger Milk (%)	00:00 (h)	03:30 (h)	05:00 (h)	06:00 (h)	08:30 (h)	11:00 (h)	12:00 (h)	15:30 (h)	16:30 (h)
0	↓	71	64	64	68	94	---	---	---
1	↓	54	58	62	70	81	70	80	89
2	↓	68	56	63	64	66	79	79	92
3	↓	52	68	69	50	56	57	68	70

TABLE 3 : The pH level in the ginger *L. paracasei* milk during incubation

Ginger Milk (%)	00:00 (h)	03:30 (h)	05:00 (h)	06:00 (h)	08:30 (h)	11:00 (h)	12:00 (h)	15:30 (h)	16:30 (h)
0	↓	6/43	6/04	5/65	5/14	4/91	4/87	4/77	4/74
1	↓	5/44	5/16	5/15	4/94	4/87	4/81	4/74	---
2	↓	5/82	5/93	5/88	5/63	5/41	5/24	5/02	4/94
3	↓	5/81	5/74	5/73	5/40	5/21	5/08	4/93	4/85

TABLE 4 : The acidity level in ginger *L. paracasei* milk during incubation

Ginger Milk (%)	00:00 (h)	03:30 (h)	05:00 (h)	06:00 (h)	08:30 (h)	11:00 (h)	12:00 (h)	15:30 (h)	16:30 (h)
0	↓	39	50	50	60	79	61	87	89
1	↓	85	75	75	76	81	75	90	---
2	↓	54	64	43	52	69	69	84	88
3	↓	43	77	58	59	78	78	87	94

TABLE 5 : The PH level in the ginger *L. casei* and *L.paracasei* (together) milk within 21-day storage in the refrigerator

PH Level			
Ginger Milk (%)	7 day	14 day	21 day
0	5/55	5/4	5/47
1	5/54	5/50	5/37
2	5/35	5/33	5/29
3	5/63	5/41	5/41

TABLE 6 : The acidity level base on Dornic degree in the ginger *L. casei* and *L.paracasei* (together) milk within 21-day storage in the refrigerator

Acidity Level in Dornic degree			
Ginger Milk (%)	7 day	14 day	21 day
0	66	83	80
1	70	76	89
2	97	86	96
3	80	81	109

TABLE 7 : Growth of microbes in the ginger *L. casei* and *L.paracasei* (together) milk

Ginger Milk (%)	10^{-5} cfu/gr
0	$8/75\times10^{11}$
1	$13/75\times10^{11}$
2	10×10^{11}
3	$13/75\times10^{11}$

DISCUSSION

Probiotic cultures are described as live microbial feed supplements that improve intestinal microbial balance and are intended for maintenance of health or prevention, rather than curing of disease. The demand for probiotic foods is increasing in Europe, Japan and the U.S. reflecting the heightened awareness among the public of the relationship between diet and health.

Traditionally, the most popular food delivery systems for these cultures have been freshly fermented dairy foods, such as yoghurts and fermented milks, as well as unfermented milks with cultures added. However, in the development of functional foods, the technological suitability of probiotic strains poses a serious challenge since their survival and viability may be adversely affected by processing conditions. This is a particular concern, given that high levels cat least 10^7 per gram or mg of live microorganisms are recommended for probiotic products[3,13]. Essence medicinal plants and herbs play a significant role in the human life and have been very popular for long among the Iranian[4]. The ginger as a medicinal plant, has extensive effects of which the most notable include arthritis, rheumatism sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious disease and helminthiasis[5].

The aim of the present study, the effects of ginger on the growth of the bacteria *Lactobacillus casei* and *Lactobacillus paracasei* (together) in probiotic milk sere investigated. The acidity, pH and survival of the bacteria in the ginger probiotic milk were evaluated at 2 h intervals till reaching 42 °Dornic acidity degrees for milk in the incubator at 38 °C and also within 21 day period of storage in the refrigerator.

The probiotic ginger milk %0 (the control sample) in the sample containing *Lactobacillus casei* reached 84-87 °Dornic acidity much earlier than other samples, which was transferred to a refrigerator and stored at 2 °C. So, this sample had the most effect on the growth of bacteria during incubation. The sample with %2 ginger and subsequently the samples with %1 and %3 ginger and finally the sample containing both bacteria, *Lactobacillus casei* and *Lactobacillus paracasei*, reached 84-87 °Dornic. So, the *Lactobacillus casei* ginger milk %3 had a minimal effect on the growth of bacteria during incubation.

The probiotic ginger milk %1 in the sample containing *Lactobacillus paracasei* reached 89 °Dornic acidity earlier than others, which was transferred to a refrigerator and stored at 2 °C. So, this sample had the most effect on the growth of bacteria during incubation. The sample with %3 ginger and subsequently the control sample and the sample with %2 ginger and finally the sample containing both bacteria, *Lactobacillus casei* and *Lactobacillus paracasei* reached 89 °Dornic. So, the *Lactobacillus paracasei* milk sample %2 had a minimal effect on the growth of bacteria during incubation.

During the 21 days storage of milk samples containing both bacteria, *Lactobacillus casei* and *Lactobacillus paracasei*, in the refrigerator, the acidity levels in the sample with %2 ginger was higher than others, and subsequently the samples with %3, %1 and %0 (the control sample) were higher, respectively. So, during refrigeration the control sample had the most persistence. The sample milk containing %2 ginger had a minimal persistence.

In the direct counting method of bacteria, maximum number of microbes (the mixture of *Lactobacillus casei* and *Lactobacillus paracasei*) were observed in the milk sample with %1 and %3 ginger and subsequently the sample with %2 ginger and finally the control sample were more, respectively.

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 yielded and incubation time for the yoghurt with malt and soya decreased[7].

A study investigating 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 yoghourt[6].

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 probitic milk and yoghurt[8].

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In other 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 milk[9].

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