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Lactobacillus acidophilus count of synbiotic dahi with addition of inulin and raffinose with different concentration of culture

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Abstract
The count of *Lactobacillus acidophilus* strain V_2 has significant variation among different treatment. The count of treatment In_1C_2 and In_1C_3 were at par with each other. The treatment In_1C_2 shown microbial counts of 195.00×10^6 cfu/g by taking in to consideration the cost of prebiotic and culture, treatment In_1C_2 was selected for further experiment. As same the RG_2C_2 having *Lactobacillus acidophilus* count 183×10^6 cfu/g was selected for further experiment.

Keywords: *Lactobacillus acidophilus*, inulin, raffinose, synbiotic

Introduction

With the growing interest in self-care and integrative medicine coupled with our health-embracing baby boomer population, recognition of the link between diet and health has never been stronger. As a result, the market for functional foods, or foods that promote health beyond providing basic nutrition, is flourishing. Within the functional foods, is the small but rapidly expanding arena of probiotics – live microbial food supplements that beneficially affect an individual by improving intestinal microbial balance. Dave and Shah (1997) [3] reported that several health benefits have been associated with the consumption of fermented foods, particularly dairy products. Certain of these health benefits are attributed to beneficial probiotic bacteria included in these fermented products. Attention, therefore, has been directed towards the inclusion of probiotic cultures in fermented products to promote health and nutritional benefits derived from the consumption of such products (Dave and Shah, 1997) [3]. The lactobacilli produce lactic acid during fermentation, which usually destroys or suppresses other less beneficial disease-causing bacteria (Chaitow and Trenev, 1990) [1]. Other claimed benefits of probiotic intake include prevention and treatment of infantile diarrhea, travelers' diarrhea, antibiotic induced diarrhea, colon cancer, constipation, hypercholesterolemia, lactose intolerance, vaginitis and intestinal infections (Franz *et al*., 2003) [4].

On the other hand, prebiotics are non-digestible food ingredients that affect the host by selectively targeting the growth and / or activity of probiotic bacteria in the colon, and thus have the potential to improve health (Marchand and Vandenplas, 2000) [6]. Regular intake of prebiotic non-digestible complex carbohydrates (bifidogenic substances) can enhance the presence of probiotic bacteria, particularly bifidobacteria, in the gastro-intestinal tract (GIT) of the host. Subsequently, there is greater protection against infection by keeping the GIT relatively acidic. Potential benefits of prebiotic intake include reduction of cholesterol absorption, control of constipation, bioavailability of minerals and reduction in blood glucose levels when used to replace sucrose in diabetic diets (Niness, 1999) [7]. A functional food product is one that can positively influence one or more functions in the body, beyond its nutritional properties (Marchand and Vandenplas, 2000) [6].

A combination of prebiotics and probiotics results in a functional product referred to as a synbiotic product. Advantages of developed symbiotic products are due to their potential synergistic effects in the host consumer. The case in point is that, upon regular consumption of a synbiotic product, there is implantation and facilitation of growth of newly ingested probiotic bacteria, promotion of growth of existing probiotic bacteria as well as improvement in survival (Niness, 1999) [7]. A synbiotic is therefore, a combination of a probiotic and prebiotic in the same product.

The advantages are that a commercial probiotic with known benefits can be used and the prebiotic aids the establishment of the organism in the complex gut environment (Kolida *et al.*, 2010) [5].

The production of fermented milk products is rapidly increasing in all major developing countries of the world. The fermented milk products like dahi, cultured butter milks, *acidophilus* milk, kefir, lassi etc. being manufactured in various countries. Fermented milks are known throughout the world for their taste, nutritive values and therapeutic properties. Dahi is one of the important Indian fermented milk product. The importance of dahi in a daily diet of Indian people has been well recognized from the vedic times. Its curative effect against gastro-intestinal disorder has been utilized by many civilizations (Sinha and Sinha, 2000) [9].

Material and methods

The composite samples of cow milk required for research were obtained from the herd maintained at the Research-Cum-Development Project on Cattle, Department of Animal Science and Dairy Science, Mahatma Phule Krishi Vidyapeeth, Rahuri. The prebiotic inulin and raffinose were purchase from Dodal Enterprizes, Aurangabad (M.S.).

The samples of synbiotic dahi were stored at refrigerated temperature for 7 days. The samples of stored synbiotic dahi were analyzed for their chemical and microbiological quality over a period of 7 days. The samples were analyzed for chemical and microbiological quality by standard analytical procedures. Experimental data was tabulated and analyzed with the help of Factorial Completely Randomized Design (FCRD) and Completely Randomized Design (CRD) method. The treatment combinations are as follows.

Starter Culture, Its Maintenance and Propagation

The freeze dried probiotic pure cultures of *Lactobacillus acidophilus* were obtained from the National Dairy Research Institute, Karnal, Haryana and SMC College GAU, Anand. The cultures were maintained separately in sterilized reconstituted skim milk test tubes.

The sterilized skim milk test tubes were separately inoculated with these cultures and incubated at 37 ^{0}C for 8 hr and thereafter stored at refrigerated temperature. In order to keep these cultures active, they were propagated once in a week. (Shankar 1975) [8].

Preparation of Synbiotic Dahi

The synbiotic dahi was prepared by using the procedure prescribed by De (2008) [2] with some minor modifications. The flow diagram for preparation of synbiotic dahi is depicted in Fig. 1.

Receiving milk
↓
Preheating (35-40 ^{0}C)
↓
Filtration/clarification
↓
Standardization (3.5 % fat)
↓
Pasteurization (90 ^{0}C/15 min.)
↓
Cooling (30-35 ^{0}C)
↓
Addition of prebiotics (1 % and 1.5 %, of milk w/v)
↓
Addition of culture of *Lb. acidophilus*: Strain 1, 2 and 3 separately (@ 1.5 %)
↓
Proper mixing
↓
Packaging in polystyrene cup
↓
Incubation (37^0 C/8 hrs.)
↓
Synbiotic dahi
↓
Cooling and stored at refrigerated temp.

Fig. 1: Preparation of synbiotic dahi

Result and discussion

In the present investigation efforts were made for preparation of synbiotic dahi from cow milk and assessed *Lactobacillus acidophilus* counts of synbiotic dahi with addition of inulin and raffinose with different concentration of culture. The results obtained have been assessed in Table 1 and 2.

1. *Lactobacillus acidophilus* counts of synbiotic dahi with addition of inulin with different concentration of culture

The perusal of data from Table 1 revealed that, the counts of *Lactobacillus acidophilus* strain V_1 has significant variation among different treatments. Counts of Treatment In_1C_2 and In_1C_3 were at par with each other. In_1C_2 *Lactobacillus* count was 96.33 x 10^6 cfu/g. Also the counts of treatment In_2C_2 and In_2C_3 were at par. By taking into considering the cost of prebiotic and culture, treatment In_1C_2 was selected for further experiment. The treatment In_3C_2 and In_4C_2 did not shown much variation in the count and hence they were rejected.

Table 1: *Lactobacillus acidophilus* count of synbiotic dahi with addition of inulin with different concentration of culture

Treatments	*Lactobacillus acidophilus* counts (10^6 cfu/g)		
	V_1	V_2	V_3
In_1C_1	85.00^{a}	192.67^{a}	186.00^{a}
In_1C_2	96.33^{b}	211.00^{cd}	195.00^{bc}
In_1C_3	98.00^{b}	213.67^{cd}	195.33^{bcd}
In_1C_4	103.33^{cd}	224.67^{fgh}	199.33^{bcde}
In_2C_1	99.67^{bc}	203.67^{b}	194.00^{bc}
In_2C_2	109.00^{ef}	219.33^{ef}	201.00^{cde}
In_2C_3	111.67^{fg}	222.00^{fgh}	202.33^{de}
In_2C_4	113.67^{ghi}	227.67^{hij}	204.67^{ef}
In_3C_1	104.67^{d}	207.33^{bc}	193.67^{b}
In_3C_2	114.33^{ghi}	221.00^{efg}	205.00^{efg}
In_3C_3	114.67^{ghi}	225.00^{fghi}	210.33^{fgh}
In_3C_4	112.67^{fgh}	230.33^{ij}	218.00^{ok}
In_4C_1	109.33^{f}	215.67^{de}	195.67^{bcd}
In_4C_2	116.67^{hij}	224.00^{fghi}	212.00^{ghi}
In_4C_3	118.67^{j}	225.33^{ghi}	216.67^{hi}
In_4C_4	117.67^{ij}	232.33^{j}	226.00^{j}
Mean	107.83	218.45	203.44
S.E. ±	1.44	2.01	2.50
C.D. at 5%	4.14	5.50	7.20

Where, In_1- 1 % inulin C_1- 1 % Probiotic culture
In_2- 1.5 % inulin C_2- 1.5 % Probiotic culture
In_3- 2 % inulin C_3- 2 % Probiotic culture
In_4- 2.5 % inulin C_4- 2.5 % Probiotic culture

In_2C_2 having *Lb. acidophilus* counts 109 x 10^6 the counts of strain V_2 has shown significant variation among different treatments. The counts of treatment In_1C_2 and In_1C_3 were at par with each other. In_1C_2 had microbial count of 211.00 x 10^6 cfu/g. By taking into consideration the cost of prebiotic and culture, the treatment In_1C_2 was selected for further experiment. Also treatment In_2C_2 and In_2C_3 were at par for microbial counts. Similarly the treatment In_3C_2 and In_4C_2 showed no much variation in the microbial counts. So as to minimize the cost, treatment In_3C_2 and In_4C_2 were rejected for further experiment.

The perusal of data from Table revealed that, the counts of *Lactobacillus acidophilus* strain V_3 has shown significant variation among different treatments. The counts of treatment In_1C_2 and In_1C_3 were at par with each other. The treatment In_1C_2 shown microbial counts of 195.00 x 10^6 cfu/g. By taking into consideration the cost of prebiotic and culture, treatment In_1C_2 was selected for further experiment. Also treatment In_2C_2 and In_2C_3 were at par for microbial counts. The treatment In_3C_2 and In_4C_2 shown not so much variation of the microbial count. So, to minimize the cost, In_3 and In_4 were rejected for further experiment.

2. *Lactobacillus acidophilus* count of synbiotic dahi with addition of raffinose with different concentration of culture

The perusal of data from Table 2 revealed that, the counts of *Lactobacillus acidophilus* strain V_1 has shown non-significant variation among different treatments. Treatment Rf_1C_2 and Rf_2C_2 shown microbial count 92.33 and 94 x 10^6 cfu/g, respectively. Therefore, treatnents Rf_1C_2 and Rf_2C_2 were selected for further experiment. The microbial count of treatments Rf_3C_3, Rf_3C_4, Rf_4C_3 and Rf_4C_4 were 98.33, 100, 103 and 106 x 10^6 cfu/g, respectively but these were not much varied from treatments Rf_1 C_2 and Rf_2 C_2. Hence, by taking into consideration the cost of prebiotic and culture and sensory qualities these treatments did not selected for further experiment.

Table 2: *Lactobacillus acidophilus* counts of synbiotic dahi with addition of raffinose with different concentration of culture

Treatments	*Lactobacillus acidophilus* count (10^6 cfu/g)		
	V_1	V_2	V_3
Rf_1C_1	74.33	154.00^{a}	155.00
Rf_1C_2	92.33	179.00^{de}	173.67
Rf_1C_3	95.67	183.00^{ef}	172.33
Rf_1C_4	96.00	187.00^{fgh}	172.67
Rf_2C_1	76.00	166.00^{b}	165.67
Rf_2C_2	94.00	183.00^{ef}	176.00
Rf_2C_3	98.00	184.00^{fg}	175.67
Rf_2C_4	101.00	188.00^{gh}	179.00
Rf_3C_1	72.67	172.00^{c}	166.00
Rf_3C_2	93.00	186.00^{fg}	179.00
Rf_3C_3	98.33	191.00^{hi}	181.00
Rf_3C_4	100.00	195.00^{ij}	181.00
Rf_4C_1	80.00	177.00^{d}	171.67
Rf_4C_2	99.00	193.00^{i}	179.00
Rf_4C_3	103.00	199.00^{j}	183.00
Rf_4C_4	106.00	203.00^{k}	188.00
Mean	92.43	183.75	174.92
S.E. ±	1.50	1.50	2.58
C.D. at 5%	N.S.	4.32	N.S.

Where, Rf - Raffinose C- Probiotic culture
Rf_1- 1 % Raffinose C_1- 1 % Probiotic culture
Rf_2- 1.5 % Raffinose C_2- 1.5 % Probiotic culture
Rf_3- 2 % Raffinose C_3- 2 % Probiotic culture
Rf_4- 2.5 % Raffinose C_4- 2.5 % Probiotic culture

The perusal of data from Table 2 revealed that counts of *Lactobacillus acidophilus* strain V_2 has shown significant variation among different treatments. Treatment Rf_1C_2 and Rf_1C_3 were at par with each other. Rf_1C_2 shown microbial count 179 x 10^6 cfu/g. By taking into consideration the cost of prebiotic and culture, Rf_1C_2 was selected for further experiment. Also, treatment Rf_2C_2 and Rf_2C_3 were at par for microbial count. Hence, Rf_2C_2 having *Lb. acidophilus* count 183 x 10^6 cfu/g was selected for further experiment.

The treatment of Rf_3C_2 and Rf_4C_2 did not show so much variation for microbial counts. So as to minimize the cost, Rf_3C_2 and Rf_4C_2 were excluded from the further experiment.

The perusal of data from Table 2, revealed that, the counts of *Lactobacillus acidophilus* strain V_3 has non-significant variation among different treatments. Treatment Rf_1C_2 and Rf_2C_2 shown microbial counts of 173.67 and 176.00 x 10^6 cfu/g, respectively. Rf_1C_2 and Rf_2C_2 was selected for further experiment. The microbial counts of treatments Rf_3C_3, Rf_3C_4, Rf_4C_3 and Rf_4C_4 were 181.00, 181.00, 183.00 and 188.00 x 10^6 cfu/g, respectively but they did not differ much from the counts of treatments Rf_1C_2 and Rf_2C_2. Hence, by taking into consideration of cost factor and sensory evaluation, these treatments were not included in further experiment.

Study conducted under Phase- I indicate that lower levels (1 and 1.5%) of both the prebiotics (Inulin and Raffinose) in combination with all three strains of *Lactobacillus acidophilus* secured higher sensory score for all the sensory parameters. Therefore, next experiment trials under Phase- II were carried out for including 1 and 1.5 per cent levels of both the prebiotics and three strains of *Lactobacillus acidophilus* for preparation of symbiotic dahi.

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