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Microbiological study of synbiotic fermented whey drink

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ABSTRACT : The present investigation was carried out for formulation of functional probiotic/synbiotic whey drink with orange juice and also to study their microbiological. Whey based functional foods were in two different forms, A [whey + sugar @ 10 % (w/v) + orange juice@ 10 % (v/v)], B [A + inulin@ 3 % (w/v)], and inoculated with probiotic culture *Lb.rhamnosus* @ 2.0% v/v. The two blends (A and B) developed was subjected to microbiological, initially for the fresh (0 day) and upto 28 days of stipulated refrigerated storage at an interval of 7 days at 4 ± 1°C. During refrigerated storage for 28 days, *Lactobacillus* count remained well above 10^8 cfu/ml for both the samples (A and B). The product had no yeast and mold count as well as coliform count throughout the refrigerated storage period. These products can surge ahead in market as appealing functional fermented whey drink for consumers as an alternative of carbonated soft drinks and give health benefits due to presence of probiotic culture, inulin, whey proteins and other whey constituents as well as orange fruit juice.

KEY WORDS : Synbiotic whey drink, Orange juice, Inulin, Microbiological, *Lactobacillus* count.

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INTRODUCTION

Whey is a by-product obtained from the manufacture of products such as paneer, chhana, cheese, shrikhand etc. It is considered to be one of reliable sources of a biologically active proteins, carbohydrates and minerals. Whey constitutes about 80 to 90 per cent of the volume of milk that is used for production of paneer, chhana, cheese and casein. It retains about 45 to 55 per cent of the milk nutrients comprising serum proteins, lactose, minerals and vitamins and is not yet fully utilized in various food formulations (Khamrui and Rajorhia, 1998). The world production of whey is about 125 million tones (2007), in which about 68 per cent is produced in European countries and 24 per cent in North America. In the absence of systematic survey/statistics, the predicted value for whey production in India is estimated at 4.84 million tones per annum (Raju *et al.*, 2005).

Most of the developed countries have stringent laws for the treatment of whey prior to disposal into sewage system. In India also, the more recent enforcement of strict environmental regulations has compelled dairy industry to reappraise waste management in general, especially in whey disposal (Gandhi, 1989 and Khamrui and Rajorhia, 1998a). It also acts as a source of bacteriophage contamination leading to failure of starter cultures in cheese manufacture and other fermented products. Therefore, many dairy organizations treat the whey before disposal. Durham and associates in 1997 indicated that treating five lacks litre of whey in sewage would cost $ 10,000 (Rs. 4, 70,000/-) per day for primary treatment and $ 1, 45,000 (Rs. 68,15, 000/-) for tertiary treatment (Khamrui and Rajorhia, 1998a). To overcome these problems efforts have been made to develop new

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processes for effective utilization of whey.

Various kinds of whey beverage with or without fermentations are thought in commercial operation in developed countries such as the United States and New Zealand (Zadow, 1986). Whey based fruit drinks are thirst quenching, light, refreshing, healthful and nutritious and less acidic than fruit juices. These benefits offer great marketing and profit potential for whey based fruit drinks (Gandhi, 1989; Mandal *et al.*, 1997 and Khamrui and Rajorhia, 1998b). The federal Republic of Germany in 1987 specified that whey drink must contain, in solid or liquid form, more than 51 per cent of whey constituents and may contain colouring, food stuffs with protein products and β-galactosidase. However, no such standards/specifications exist in India (Kumar, 2004). The Greek term 'Oros' (which means whey) is still evident in the terms of oroatic acid, previously known as Vitamin B13. It is higher in concentration in cow milk (8 mg/10 ml) than buffalo milk. It is an essential growth factor for *Lb. delbrueckii* ssp. Bulgaricus. When used, whey alone possesses disadvantages as it has an odd taste and liquid consistency which needs to be masked and modified. So, converting whey into a refreshing and healthy drink by growing culture of lactic acid bacteria seems to be an economically sound alternative. Metchnikoff suggested that man should consume fermented milk with *Lactobacilli* in order to prolong life. Dhole (1997) reported that *Lb. acidophilus* and Bifidobacteria in the diet will colonize the intestinal tract of man and are responsible for maintaining intestinal health.

The present definition of probiotic is "a live food microbial supplement which effects it´ s receptor in a beneficial form, through the improvment of the gut (colon) flora balance". The prebiotic foods are some types of dietary fibre as the non-digestible carbohydrates which are not digestible for the human, and which have a molecular configuration which makes them resistent to enzyme action. Samples of efficient prebiotics commercially available are fructo-oligosaccharides (FOS), inulin, lactulose and galacto-oligossaccharides.

Prebiotics are defined as non-digestible nutritional ingredients which affect positively the host, stimulating selectively the growth and the action of one or more beneficial intestinal bacteria; it improves the host´s health (Gibson and Roberfroid, 1995). Consumption of prebiotics can modulate the gut flora through the increase of the number of bifidobacteria. Inulin is a compound extracted from the root of chicory. It benefits the gut flora health and therefore, considered as prebiotic. The prebiotics of major interest are those which stimulate the growth of the gastrointestinal (GI) tract as the Bifidobacterium strains. These bacteria inhibit the growth of micro-organisms which cause diarrhoea or for an example colon cancer. Besides that the consumption of inulin by the bifidobacterium enhances a good digestion and stimulates the elimination of toxins and cholesterol. Inulin and fructo-oligosaccharides are probably the most commonly used prebiotics in the food industries. Reduces the risk of osteoporosis because provides the calcium bioavailability (Holzapfel and Schillinger, 2002). Several studies indicate that inulin is selectively associated to the bifidobacteria growth in the human gut. The combination of prebiotics and probiotics results in the concept of synbiotics.

The aim of present study is to formulate new functional synbiotic whey drink, using *L.acidophilus* and inulin, which will be beneficial to the lactose intolerant peoples and sympathizers.

Advantages of inulin :

Selective fermentation of prebiotics by such micro-organisms must result in a healthier composition of the gut microflora and induce lumenal or systemic effects, beneficial to the host (Gibson and Roberfroid, 1995 and Roberfroid, 2001). All fibre acts as a prebiotic in varying degrees. Feeding fibre benefits our intestinal eco-system, improve immunity and overall health (Macfarlane and Cumming, 1999; Schley and Field, 2002 and Tungland and Meyer, 2002) Several excellent review articles by various workers (Gibson and Fuller, 2000; Steer *et al*., 2000 and Kumar *et al*., 2005) are available on the probiotics and prebiotics, their health effects, mode of action, growth and survival in GIT, quality assurance criteria and safety including future prospects. Prebiotics, especially inulin, helps in improvement of mineral absorption like zinc and copper (Scholz-Ahrens *et al*., 2001; Scholz-Ahrens and Schrezenmeir, 2002; Chen and Chen, 2004 and Coudray *et al*., 2006), improve calcium bioavailability (Manning and Gibson, 2004 and Weaver, 2005) results in better lipid metabolism, improved immunostimulation and helps in lactose intolerance (Roberfroid, 2000). Studies have shown their protective role in preventing colon cancer (Wollowski *et al*., 2001; Manning and Gibson, 2004; Leu *et al*., 2005 and Van Loo *et al*., 2005) and coronary heart diseases (Roberfroid, 2005).

Probiotic agents have been revealed to have

significant clinical beneficial effects in the prevention and management of gastrointestinal and non-gastrointestinal conditions (Saavedra and Tschernia, 2002). Prebiotics also aids in treating various disorders *viz*., necrotizing entrocolitis, traveller's diarrhoea, allergic colitis (Davidson and Butler, 2000; Steer *et al*., 2000 and Macfarlane and Cumming, 2002) inflammatory bowel diseases (Kanauchi *et al*., 2003 and Guarner, 2005) obesity (Roost, 2005) and in pediatrics gastrointestinal disorders, by increase in biomass of probiotics and stool bulking.

Objectives :

–To formulate functional synbiotic whey drink containing probiotic culture (*Lb. rhamnosus* MTCC 5462 previously known as *Lb. acidophilus* V3) and prebiotic (inulin) and also to analyze microbial analysis of fresh product, as well of stored product at the regular interval of 7 days till 28th days of the refrigerated storage (4 ±1 °C).0

–To evaluate the changes in probiotic population after manufacture and during storage.

–To check the presence of aerial contaminants like yeast and mold and to evaluate index of hygiene by captivating coliform count.

–To check the shelf-life of the product using refrigerated storage study determined at refrigerated temperature of 4 ±1 ºC.

MATERIAL AND METHODS

The work was carried out in the Department of Dairy Microbiology SMC College of Dairy Science, Anand. The raw materials, which were used during the course of study along with their sources are delineated here under.

The fresh cow milk was collected from Livestock Research Station, Anand for manufacturing of synbiotic whey drink. Inulin was added @ 3 % (w/v) for the formulation of the final product. Oraftii Ltd, Belgium, supplied the inulin having trade name Raftiline.Sugar For fortification of synbiotic whey drink with sweetener high quality sugar, free from an impurity was purchased from local supplier.

Probiotic culture and its maintenance :

The culture used in the present study was *Lb. rhamnosus* MTCC 5462 obtained from the culture Collection of Dairy Microbiology Department, SMC College of Dairy Science, Anand. The culture @ 2 % v/v was propagated in sterilized skim milk (10 % T.S., Sagar skim milk powder, Dudhsagar) for 16 h and in sterile whey for 12 h and stored at 5 ± 2 °C. The transfer was given every week during the course of the study.

Preparation of synbiotic whey drink :

A [whey + sugar @ 10 % (w/v) + orange juice@ 10 % (v/v)]

B [A + inulin@ 3 % (w/v)], and inoculated with probiotic culture *Lb. rhamnosus* @ 2.0% v/v. (Fig. A).

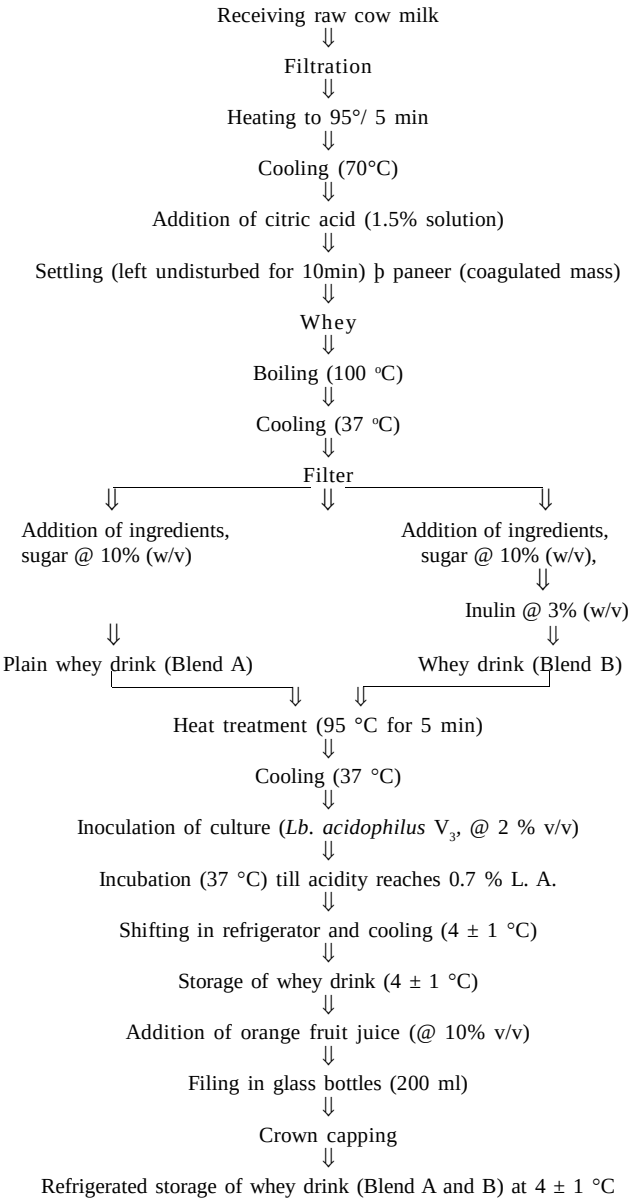

Fig. A: Flow chart for preparation of synbiotic whey drink

Microbiological analysis of synbiotic whey drink :

Preparation of samples for microbiological analysis of whey drink:

After fruit juice addition to synbiotic whey drink on the 0 day, a 11 ml from each treatment taken for conducting *Lb. acidophilus* count (LAB), coliform count (CC) and yeast and mould count (YMC). Similarly, thereafter, at an interval of 7 days, upto 28 days of storage.

After mixing of whey drink, 11 ml of sample was weighed aseptically in a sterile phosphate buffer flask to prepare 1:10 dilution. After thorough mixing, 1 ml of diluent from 1:10 was transferred in to 9 ml sterile phosphate buffer tube to make 1:100 dilutions. It was further appropriately diluted using 9 ml sterile phosphate buffer to make required dilutions. One millilitre of diluent, from suitable dilutions was poured aseptically in sterile petriplates in triplicate. The time elapsed between preparations and pouring of appropriate dilution in sterilised petri plates normally did not exceed 15 min.

Preparation of Agar plates for Lactobacilli count:

Lb. acidophillus count of inoculated whey drink samples was determined as per the method described by De man *et al.* (1960). Petri plates containing the appropriate dilutions were poured with melted and cooled MRS agar medium at around 45 °C, mixed properly and allowed to solidify. A second layer of same agar (4-5 ml) was overlaid in each plate. The Petri plates were then incubated at temperature of 37 °C and colonies formed on the medium were counted after 48 h and count was recorded as *Lactobacillus* count cfu/ml.

Preparation of agar plates for coliform count:

The freshly prepared, unsterilized Violet Red Bile Agar (VRBA) was melted and cooled at around 45 °C. Around 12-15 ml was added in plates containing 1 ml of appropriate dilution in triplicate, mixed well and allowed to solidify. A second layer of same agar (4-5 ml) was overlaid in each plate. Typical colonies of coliform bacteria were counted and expressed as cfu/ml after 24 h of incubation at 32 °C. Plates showing no colony growth, were incubated for a further period of 24 h. Plates were then examined for typical colonies of coliform bacteria (dark red colonies having a diameter of > 0.5 mm) and counted (Indian Standards, IS: 5401, 1969). Similar procedure was also given by Marshall (1992) for enumeration of coliforms from food sources.

Preparation of agar plates for yeast and mold count:

According to Indian Standards, IS: 5403, (1969) procedure was followed for the enumeration of yeast and mold. The Petri plates containing the diluted samples were poured with melted and cooled potato dextrose agar medium at around 45 °C (adjusted to 3.5 pH using 10 % sterile tartaric acid solution). Mixed properly and allowed to solidify. The plates were incubated at temperature 22-25°C for 3-5 days and the yeast and mold count was recorded as cfu/ml. Comparable procedure is also given for enumeration of yeasts and molds from food sources by Marshall (1992).

RESULTS AND DISCUSSION

The results of the present study as well as relevant discussions have been presented under following sub heads:

***Lactobacilli* count :**

Changes in *Lactobacilli* counts of synbiotic whey drink (A and B) during refrigerated storage (4 ± 1 °C) are Fig. 1 and Table 1. Indicates mean values and Table 2. Shows ANOVA table for the same. Both the freshly made (0 day) whey drink samples had lactobacilli count varying from log values 8.37 to 8.85 (Table 1). The statistical data showed that the treatments were non-significant. During refrigerated storage of the samples (0 to 28 days), in case of sample A as well as B there was slight decline in *Lactobacillus* count. In case of sample A and B *Lactobacilli* count was highly significant at the end of 28 days in comparison to fresh samples.

While in case of sample B during the storage period (14, 21, 28) *Lactobacilli* count were highly significant (P<0.01). Nevertheless, in the synbiotic whey drink samples

Table 1: Changes in *Lactobacilli* count of functional probiotic/synbiotic whey drink with orange juice during refrigerated storage (4 ± 1°C)

Storage period (Days)	0	7	14	21	28	Treatment mean
A	8.8500	8.5275	8.4950	8.3850	8.3925	8.5300
B	8.7700	8.7700	8.5000	8.3775	8.3725	8.5580
Period mean	8.8100	8.6488	8.4975	8.3813	8.3825	
Total :341.76				General mean: 8.544		

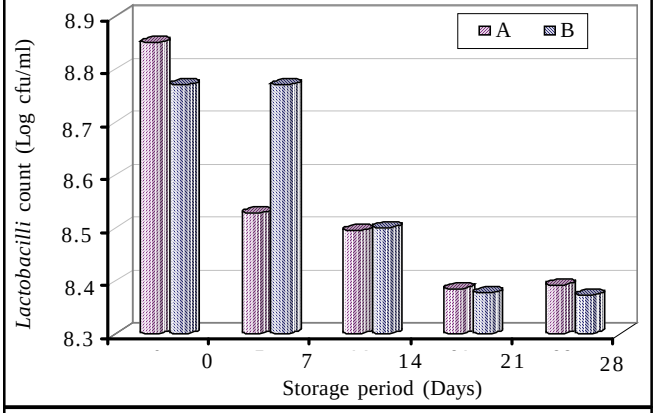

Fig. 1 : Changes in *Lactobacilli* count of functional probiotic/ synbiotic whey drink during refrigerated storage (4± 1°C)

A and B the count of *Lactobacilli* remained well above10^8 cfu /ml even after the end of refrigerated storage for 28 days. The interaction (T x I) between treatments (T) and the storage period (I) was also non- significant. This indicates that there was not much difference in *Lactobacilli* counts during storage period for the two blends.

Dhole (1997) prepared whey beverage containing selected probiotic cultures using *Lb. acidophilus* and *B. adolescentis* (@ 5%, v/v). Cheese whey (with 1:1 proportion of sweet and salted cheddar cheese whey) with incorporation of tomato juice and sugar and fermented. The results showed reduction in the count but they remained around of 10^6- 10^7 cfu/ml of the finished product. *Lb. acidophilus* showed 42 x 10^7 cfu/ml and *B. adolescentis* showed 57.5 x 10^7cfu/ml and in combination the count increase in cell population in the range of 50 x 10^7 to 134.9 x 10^7 cfu/ml after 8 h.

Coliform counts :

The presence of coliform bacteria in dairy products is suggestive of insanitory conditions or practices followed during production, processing and storage (Speck, 1984). The confirmation of presence of faecal coliforms in the products, further, indicates probable and alarming presence of potent human pathogens too. In the present work to adjudge mainly the extent of sanitary practices followed during manufacturing, or otherwise, the coliform count of synbiotic whey drink samples was carried out.

It was found that during entire course of study, the coliforms in both fresh blends initially, as well as during all the intervals at refrigerated storage periods was absent in 1 ml whey drink samples. Absence of coliform count indicates that the hygienic conditions adapted during manufacturing and storage of whey drink samples as well as the sanitation of the bottles and caps.

Yeast and mold count :

Yeasts and molds perhaps, are one of the most important groups of microbes present among several other groups of spoilage microflora in fermented milk products, including whey drinks. Yeasts and molds are mainly contaminated to the whey drink samples through air (Tamime and Robinson, 1999).

It was observed that yeast and mold count for both the blends of whey drink *i.e.* A and B, were absent in 1 ml whey drink during the entire storage period of 0 to 28 days. This is the indication of good aerial sanitation as well as hygienic conditions during manufacturing and storage of synbiotic whey drink.

Conclusion :

Therapeutic and health promoting properties of whey were acclaimed already by Hippocrates - father of modern medicine in 460 B. C. Over the years, modern man also realized the importance of using whey in everyday nutrition. The functional probiotic/synbiotic whey drink with orange juice was developed for the first time in the world. The products had shelf-life of 28 days at 4 ± 1°C. Without any microbial changes, At the end of storage period of 28 days, both the products were acceptable with a viability of well above 10^8 cfu/ml. These

Table 2 : ANOVA table for *Lactobacilli* count (cfu/ml) of functional probiotic/ synbiotic whey drink with orange juice during refrigerated storage (4 ± 1 °C)

Source	DF	SS	MS	F Cal	F Tab (1%)	F Tab (5%)	SEm	C.D.(P=0.05)	Test
Treatment	1	0.008	0.008	0.133	7.56	4.170	0.055	-	NS
Interval	4	1.092	0.273	4.550	4.02	2.690	0.087	0.250	**
T x I	4	0.123	0.031	0.515	4.02	2.690	0.122	-	NS
Error	30	1.796	0.060			CV%: 2.86			

** indicate significance of value at P<0.01, DF- Deviation factor, SS-Sum of square, MS-Mean square, S.E.± –Standard error mean , CD-Critical difference, NS=Non- significant

products can surge ahead in market as appealing functional whey drink for consumers as an alternative of carbonated soft drinks and give health benefits due to presence of probiotic culture, inulin, whey proteins and other whey constituents as well as orange fruit juice.

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