EFFECT OF SYMBIOTIC PROPERTIES ON VIABILITY OF PROBIOTIC ORGANISMS IN DESIGNER YOGHURTS

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Living longer with health and fitness is the mind of most people and food is one of the most important facets of a healthy life. It seems very likely that concept of "designer foods" which enables the consumer to exercise a level of self-health maintenance, will significantly influence food and drink manufacturer. In this context the current study is focused on design of yoghurt (set yoghurt and yoghurt drink) with symbiotic (combination of prebiotics and probiotics) properties and assess the efficacy of designer yoghurt to combat overweight. Set yoghurt was designed by optimizing with Response Surface Methodology and yoghurt drink was designed with Factorial Research Design. The optimized samples were treated as experimental set yoghurt and experimental yoghurt drink. For each experimental sample two control samples i.e., positive control and negative control samples were also designed. All samples were analysed for viability of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The results showed both experimental samples with symbiotic properties had more probiotic activity compared with control samples. Compared with experimental yoghurt drink experimental set yoghurt had more viability of both probiotic organisms. Whereas the growth of *Streptococcus thermophilus* was more compared with *Lactobacillus bulgaricus* in all samples.

Key words: Designer foods, prebiotics, probiotics, symbiotics, set yoghurt, yoghurt drink.

INTRODUCTION

The term probiotic is a word meaning "for life" and it is currently used to name bacteria associated with beneficial effects for humans and animals**.** Probiotics are believed to play very important roles in regulating proper intestinal function and digestion - by balancing intestinal microflora. These 'good bacteria' are considered to be "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2017). The concept of probiotics was introduced in early 20th century by Elie Metschnikoff, it however gained momentum only recently with considerable and significant advances in functional and health food market across the world. India is also fast emerging as a potential market for probiotics in food.

The range of food products containing probiotic strains is wide and still growing. The main products existing in the market are dairy-based ones including fermented milks, cheese, ice cream, buttermilk, milk powder, and yoghurts, the latter accounting for the largest share of sales. Non-dairy food applications include soy based products, nutrition bars, cereals, and a variety of juices as appropriate means of probiotic delivery to the consumer. The factors that must be addressed in evaluating the effectiveness of the incorporation of the probiotic strains into such products are, besides safety, the compatibility of the product with the microorganism and the maintenance of its viability through food processing, packaging, and

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storage conditions. Probiotics are also available in supplement form as capsules, liquid and chewable. There are many different strains of probiotics, but the most common strains used in manufacturing of yoghurt are *Streptococcus thermophilus* and *Lactobacillus bulgaricus.*

The probiotic dose levels should be based on the ones found to be efficacious in human studies and the cfu/g of product is an important parameter. Although the information about the minimum effective concentrations is still insufficient, it is generally accepted that probiotic products should have a minimum concentration of 10^6cfu/ml or gram and that a total of some 10^8 to 10^9 probiotic microorganisms should be consumed daily for the probiotic effect to be transferred to the consumer. Furthermore, the strains must be able to grow under manufacture and commercial conditions and should retain viability under normal storage conditions (Isolauri *et al.*, 2001).

Worldwide, growing awareness of the physiological benefits of dietary fiber, among consumers, coupled with the availability of commercial dietary fiber preparations has led to the successful development of various dietary fiber fortified dairy products. The use of fibers in dairy products is also widespread: e.g. inulin introduces numerous improvements into dairy products. It improves body and mouth feel in cheese analogues or ice cream, and reduces syneresis in yoghurt and other fermented milk products (Blecker *et al.*, 2001). Dietary fiber intake provides many health benefits. Individuals with high intakes of dietary fiber appear to be at significantly lower risk for developing life style diseases like coronary heart disease, stroke, hypertension, diabetes, obesity, and certain gastrointestinal diseases.

Lifestyle means a pattern of individual practices and personal behavioural choices that are related to elevated or reduced health risk. The diseases which primarily arise from the abnormal lifestyle of a person are grouped under the term “Lifestyle Diseases”. These diseases may develop in a person due to faulty eating and living habits e.g., obesity, diabetes and hypertension etc. The World Health Organization (WHO, 2018) describes overweight and obesity as one of today’s most important public health problems, which is escalating as a global epidemic. Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and increased health problems. The problem of overweight and obesity is confined not only to adults but also being reported among the children and adolescents of developed as well as developing countries. According to a WHO report, obesity has been identified as a major cause of disability and premature deaths in less developed countries. This has been attributed to shifts in diet and lifestyle changes. The risk of many diseases including cardiovascular diseases (CVDs), hypertension, hyperlipidaemia, diabetes mellitus, and certain cancers increases many folds in association with obesity (Misra *et al.,* 2011).

Prevalence of overweight and obesity are increasing in India in recent years even though under nutrition continues to be an important public health issue (WHO, 2018). Despite the availability of a few therapeutic agents, the management of obesity is still mainly non-pharmacological (Pappachan, 2011). Physical activity and dietary modifications are the cornerstones of management of overweight and obesity.

Nowadays to control or /and prevent lifestyle disorder like obesity which is having more associated risk factors like diabetes, blood pressure, cardiovascular diseases, some type of cancers etc., there is a need to develop designer foods by incorporating more than one

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bioactive ingredient to promote various beneficial health effects. Yoghurt is the most popular fermented milk in the world, which has been readily accepted by consumers and also due to the image of the product as 'healthy'. The popularity of yoghurt has increased significantly in the last few years because of the incorporation of the probiotic microorganisms into the product that gives an extra nutritional-physiological value. Hence there is a need to manufacture food products like yoghurt with synbiotic properties. Consequently, the viability of probiotic strains during product manufacturing and storage is very important, especially, in mixed strains and/or synbiotic products. However, it is important to design probiotics, prebiotics and synbiotics that are fully compatible with formulation, processing, packaging, and distribution. The main aim of the current research is to formulate and develop designer yoghurt (set yoghurt and yoghurt drink) with synbiotic properties. Hence the present investigation has been carried out to know the effect of symbiotic properties on viability of probiotic organisms of designer yoghurts

METHODOLOGY

The research was carried out by designing two yoghurt samples (set yoghurt and yoghurt drink). For designing set yoghurt 16 formulation were made to optimize the selected factors i.e., psyllium husk fiber percentage and solid not fat percentage of milk by Response Surface Methodology (RSM). The processing parameters like psyllium husk soaking time, pasteurization time and temperature, inoculation temperature and incubation time were standardized. The set yoghurt designed based on the optimized factors (14.5 % fat and 0.68g psyllium husk powder) and standardized processing parameters (10 min psyllium husk soaking time, 63^{o} C for 30 min pasteurization temperature and time, 42^{o} C inoculation temperature and 4 hrs incubation time) was treated as experimental sample. The experimental sample (optimized) was designed with skimmed milk with 0.5 % fat, 14.5 % SNF (Solids Not Fat), and 0.68g psyllium husk powder. To know the functionality and viability of probiotic organisms of experimental sample two control samples i.e., positive control and negative control samples were designed. Positive control sample was designed with skimmed milk with 0.5 % fat, 14.5 % SNF and without addition of fiber. Negative Control sample was designed with commercially available formula (3.5 % fat and 8.5% SNF). The standardized and optimized composition of set yoghurt is presented in table 1.

Table 1: Standardized and optimized composition of set yoghurt

S.No	Ingredients	Composition		
		Experimental (set yoghurt)	Positive control (set yoghurt)	Negative control (set yoghurt)
1	Skimmed milk with 14.5% SNF	100 ml	100ml	-
2	Whole milk (3.5% fat and 8.5%SNF)	-	-	100ml
2	Psyllium husk (g)	0.68 g	-	-
3	Inoculum (%)	2 %	2 %	2 %

4	Fiber soaking time	10 min	-	-
5	Pasteurization temperature	63^0c for 30 min	63^0c for 30 min	63^0c for 30 min
6	Inoculation temperature	42^0 C	42^0 C	42^0C
7	Incubation time	4 hrs	4 hrs	4 hrs

The experimental set yoghurt designed above was used to design experimental yoghurt drink. Here also 16 formulations were made to optimize the selected factors i.e., pine apple fruit juice concentrate, stabilizer percentage by factorial research method. The yoghurt drink designed based on the optimized factors (fruit juice concentrate 40%, stabilizer percentage 0.75%) was treated as experimental yoghurt drink. To know the functionality and probiotic viability of experimental yoghurt drink two control samples i.e., positive control and negative control yoghurt drinks were designed with their respective set yoghurts. The experimental (optimized) and positive control yoghurt drink were designed with 60% respective set yoghurts, 40% of pineapple juice concentrate and 0.75% stabilizer without addition of sugar. Negative control yoghurt drink was designed with 78 % respective set yoghurt, 10 % pineapple juice concentrate, 12 % of sugar and 1 % stabilizer. The optimized composition of yoghurt drink is presented in table 2.

Table 2: Optimized composition of yoghurt drink

S.no	Ingredients	Composition		
		Experiment	Positive control	Negative control
1	Experimental set yoghurt(g)	60	--	--
2	Positive control set yoghurt (g)	--	60	--
3	Negative control set yoghurt(g)	--	--	78
4	Fruit juice concentrate (g)	40	40	10
5	Sugar (g)	--	--	12
6	Stabilizer (%)	0.75	0.75	1

Then all yoghurt (experimental, positive control and negative control) samples were enumerated for viability of probiotic organisms i.e., L.*bulgaricus* and S.*thermophilus* by using following standard protocols.

Enumeration of probiotics in designer yoghurts

All the yoghurt samples were enumerated for S. *thermophilus* and L. *bulgaricus* in triplicates by using standard methods and protocols followed in ISO (2015) methods.

Statistical Analysis

The statistical program of SPSS version.20 was applied to test the statistical constants and accordingly the results were tabulated. One way ANOVA was employed to test the levels of significant difference among the viability of probiotic organisms of three different types of designer yoghurts

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RESULTS AND DISCUSSION

Probiotic activity of set yoghurt

Probiotics are living organisms that are used as food additives with beneficial effects on the healthy body by setting microbial balance in gastrointestinal tract. The data pertaining to the viability of probiotic organisms of set yoghurt based on the L.*bulgaricus* and S.*thermophilus* of the experimental, positive control and negative control set yoghurt are presented in table 3

Table 3: Viability of selected probiotic organisms in set yoghurt

Parameter	Experimental (Mean± SD)	Positive control (Mean ± SD)	Negative control (Mean ± SD)	F value	P value	PFA standards/ g
L.*bulgaricus* cfu/g(10^8)	8.2 ± 0.2	7.0±0.01	7.5 ±0.2	33.25	0.000**	> 10^7
S.*thermophilus* cfu/g(10^8)	8.7 ± 0.2	7.3±0.15	7.6 ±0.1	62.22	0.000**	> 10^7

** Significant at 0.01 level (p<0.01) * Significant at 0.05 level (p<0.05)

The data in the table 3 shows that the growth of the L. *bulgaricus* (cfu/g(10^8)) in experimental, positive control and negative control set yoghurt were 8.2 ± 0.2, 7.0±0.01 and 7.5 ±0.02 respectively. The growth of the L.*bulgaricus* had a significant difference (P<0.01) between experimental and control samples as shown in fig.1. The growth was more in experimental sample was due to the addition of psyllium husk powder, which is a soluble dietary fiber that favours the growth of probiotic microorganisms. In between control samples, the negative control had more growth because of fat content which facilitates the growth of microorganisms.

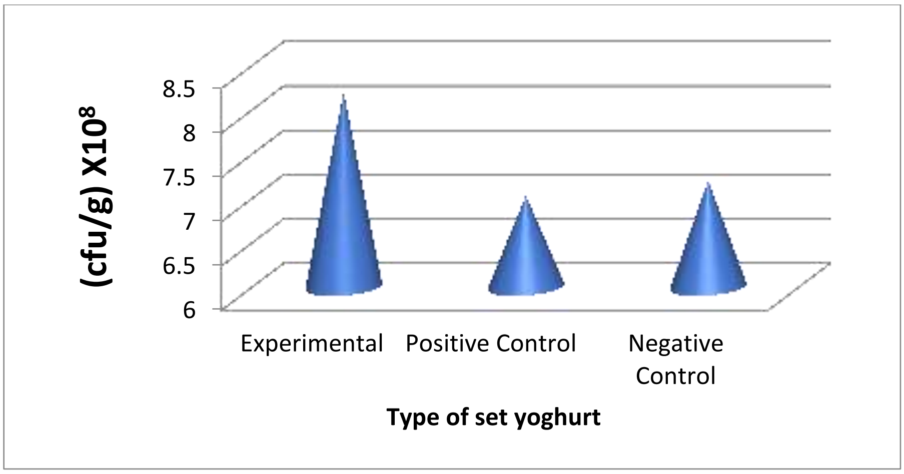

Fig.1 : L. *bulgaricus* activity of designer set yoghurt

Data also shows the growth of the S.*thermophilus* cfu/g(10^8) in experimental, positive control and negative control yoghurt drinks were 8.7 ± 0.2, 7.3±0.15 and 7.6 ±0.1 respectively. There was a significant difference (P<0.01) between experimental and control samples for the growth of S.*thermophilus* as shown in fig.2. The growth was more in experimental sample as it was due to the addition of psyllium husk powder, which is a soluble dietary fiber that favours the growth of probiotic microorganisms. In between control samples, the negative control had more growth because of fat content which facilitates

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the growth of microorganisms. The experimental yoghurt had contained acceptable level of probiotic organisms along with prebiotics (dietary fiber psyllium husk). Hence the designed experimental yoghurt had synbiotic properties.

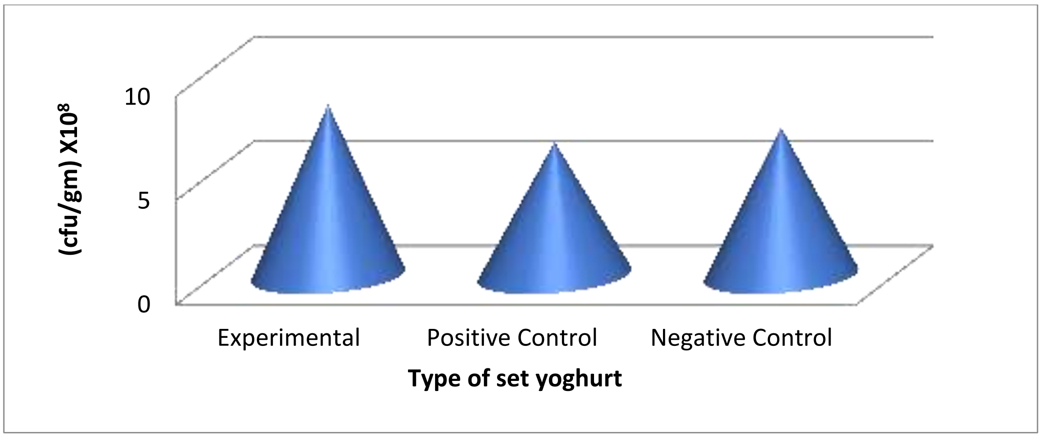

Fig.2: S.*thermophilus* of designer set yoghurt

Synbiotics are defined as mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract of the host (Andersson *et al.*, 2001). Synbiotics is where probiotics and prebiotics are used in combination to manage microflora (Fooks *et al.*, 1999).

Lactobacillus bulgaricus probiotic activity can be ascribed to its ability to produce substances with antimicrobial properties. *Lactobacilli* are known to inhibit the growth of pathogenic bacteria, possibly by producing inhibitory compounds such as organic acids, hydrogen peroxide and bacteriocins (Loessner *et al.*, 2003). S. *thermophilus* is physiologically and biochemically less versatile than other lactic acid bacteria, the reality is that this organism is actually very versatile. Research during the past two decades has revealed that S. *thermophilus* has properties that make it one of the most commercially important of all lactic acid bacteria. S. *thermophilus* is used, along with *Lactobacillu*s spp., as a starter culture for the manufacture of several important fermented dairy foods, including yoghurt and Mozzarella cheese. S. *thermophilus* has an important role as a probiotic, alleviating symptoms of lactose intolerance and other gastrointestinal disorders.

Lactic fermentation is the most important process in the manufacture of sour milk products (including yoghurt). The production time and properties of the end product depend on the qualities and activity of the starter culture. The traditional yoghurt culture is comprised of S. *thermophilus* and L. *bulgaricus*. It was found by Courtin & Francolse (2004) that these two microorganisms' association affects the production of volatile molecules involved in flavor development. The two microorganisms enter a synbiotic relationship, which means that are mutually beneficial during fermentation (Kroger *et al.*, 1976). Both microorganisms perform better in symbiosis than if grown separately. Initial pH of the milk favors the faster growth of Streptococci. Thereafter, increase in acidity favors the growth of *Lactobacilli* whose optimum pH is below 4.5. Initially, L. *bulgaricus* benefits the growth of the Streptococci by releasing the amino acids valine, leucine, histidine, and methionine from the milk proteins. For its part, S. *thermophilus* promotes the growth of *Lactobacilli* by creating minute amounts of formic acid. At least in the initial phase, the mutual stimulation of the two species in the mixed culture causes more lactic acid and aromatic compounds to be formed faster than would be the case with any of the two single cultures.

According to the study conducted by Deepika *et al.*, (2017b) who developed a srikhand and buttermilk with psyllium husk powder along with the starter culture containing S.*thermophilus* and L.*bulgaricus*. The results noted that as the concentration of psyllium husk increased, the growth of S.*thermophilus* and L.*bulgaricus* also increased. These results are in coinciding with current study. Supavititpatana *et al.*, (2010) studied the chemical, physical and microbial characteristics, and shelf-lives of corn milk and cow milk yoghurts. The counts *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were higher in corn milk yoghurt which contains fiber, than those of the cow milk yoghurt. These results are also in accordance with the results of the current study.

Although quantitative standards for yoghurt bacteria differ, as it is generally accepted that yoghurt should contain 10^7 cfu of viable bacteria (S. *thermophilus* and L. *bulgaricus*) per ml of yoghurt. According to Fadela *et al.*, (2009) who reported that during the fermentation process, the number of S. *thermophilus* is relatively higher compared to L. *bulgaricus*. Hence all the set yoghurts designed were maintained acceptable viable counts i.e. L. *bulgaricus* and S. *thermophilus* with good probiotic activity.

Probiotic activity of yoghurt drink

The data pertaining to the probiotic activity based on the L.*bulgaricus* and S.*thermophilus* of the experimental, positive control and negative control yoghurt drinks are presented in table 4.

Results shows that the growth of the L. *bulgaricus* cfu/g(10^7) in experimental, positive control and negative control yoghurt drink sample were 7.8 ± 0.07, 6.2±0.05 and 7.2 ±0.12 respectively. The growth of the L.*bulgaricus* had significant difference (P<0.01) between three samples as shown in fig.3. The growth was more in experimental sample than negative control followed by positive control. The more probiotic activity of experimental sample was due to the addition of psyllium husk powder, which is a soluble dietary fiber which favours the growth of probiotic microorganisms. In between control samples the negative control had more growth because of fat content which facilitates the growth of microorganisms.

Table 4: Probiotic activity of yoghurt drinks

Parameter	Experimental (Mean± SD)	Positive control (Mean± SD)	Negative control (Mean± SD)	F value	P value	PFA standards/ g
L.*bulgaricus* cfu/g(10^7)	7.8 ± 0.07	6.2±0.05	7.2 ±0.12	70.02	0.000**	> 10^7
S.*thermophilus* cfu/g(10^7)	8.2 ± 0.05	7.0±0.10	7.5 ±0.01	138.914	0.000**	> 10^7

****** Significant at 0.01 level (p<0.01) * Significant at 0.05 level (p<0.05)

NS - Not significant

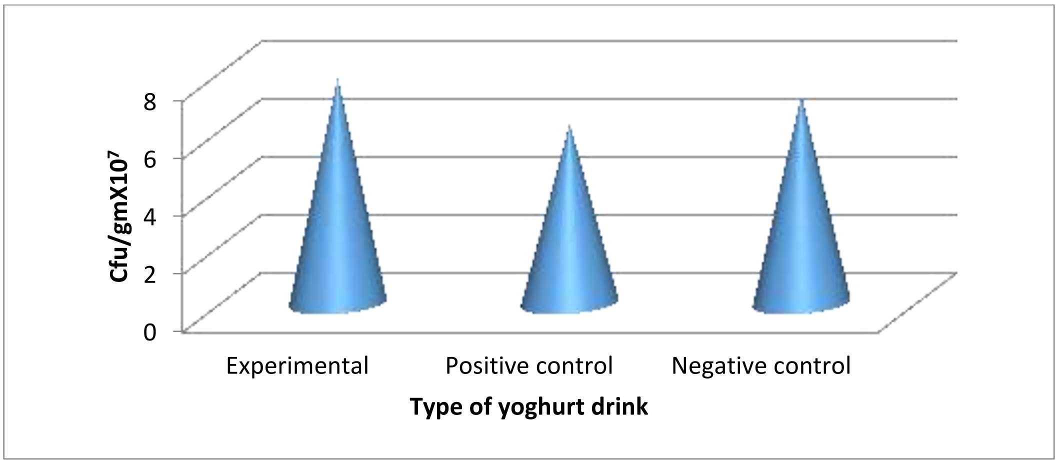

Fig. 3: L.*bulgaricus* count of designer yoghurt drink

The growth of the S.*thermophilus* cfu/g (10^7) in experimental, positive control and negative control sample were 8.2 ± 0.05, 7.0±0.10 and 7.5 ±0.01 respectively. There was a significant difference (P<0.01) between experimental and control samples for the growth of S.*thermophilus* as shown in fig.4. The more probiotic activity in experimental sample was due to the addition of psyllium husk powder, which is a soluble dietary fiber that favours the growth of probiotic microorganisims. In between control samples the negative control had more growth because of fat content which facilitates the growth of microorganisms.

The experimental yoghurt had contain acceptable level of probiotic organisms along with prebiotics (dietary fiber psyllium husk). So, the designed experimental yoghurt drink had synbiotic properties.

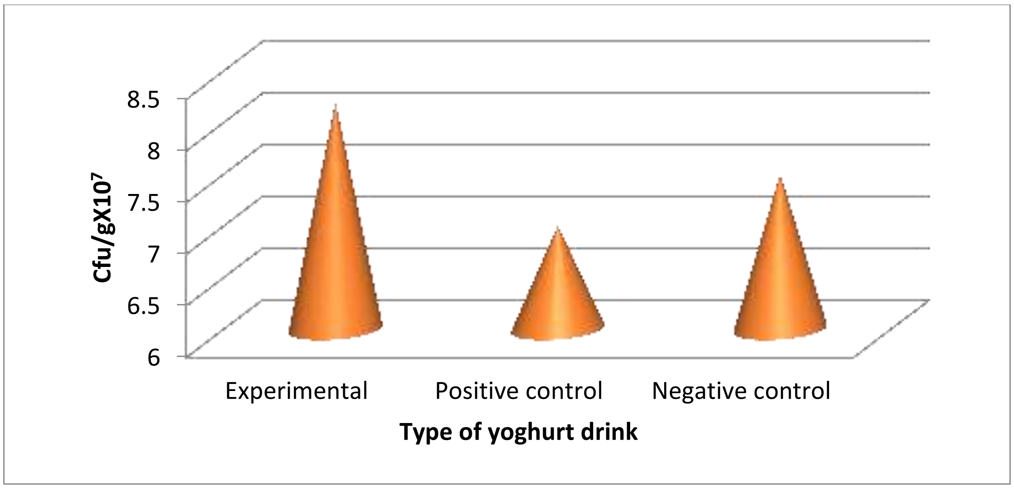

Fig. 83: S.*thermophilus* count of designer yoghurt drinks

Soluble fiber preparations have gained tremendous popularity over the years as a fiber ingredient for use in various dairy products to enhance their physiological and functional roles. In recent years, yoghurts containing probiotics have gained popularity. These products contain *Lactobacillus* and *Bifidobacterium* species at 10^6 viable cells per millilitre of the product at the time of consumption (Arunachalam, 1999). As such, there is no RDA of prebiotics, but doses in the rage of 4-20 g/d are required to show health benefits (Tuohy *et al*., 2003). Correa-Matos *et al*., (2003) reported that the milk formula with added fermentable fiber enhanced the intestinal function and reduced the severity of induced infection by *Salmonella typhimurium* in piglets.

Miremadi & Shah (2012) reviewed the effect of inulin and probiotics on colonic microbiota and efficiency of functional attributes of synbiotic foods in formulation and development of new dairy products. Recent trend for incorporating dietary fiber (DF) into yoghurt is also accompanied with the addition of the probiotic cultures (this is known as synbiotic).

Costmagna & Rossi (1977) was found that the wheat bran addition did not retard the growth of *Streptococcus thermophilus* and shows beneficial effects on the growth of *Lactobacilus bulgaricus*. Ozer *et al*., (2005) observed that the addition of inulin caused a substantial increase in the cell counts of *Bifidobacterium bifidum* BB-02, being about 4.6 and 7.5 fold for the inulin added samples at levels of 0.5% and 1.0%, respectively

Yoghurt drink is a fermented dairy product produced by the synbiotic microbial action of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The observation of synbiotic relationship between these two microorganisms was first reported by Orla-Jenson, (1931). *Lactobacillus bulgaricus* is the new nomenclature for L. *bulgaricus*. Because of the high phenotypic and genomic similarities between L. *delbrueckii*, L. *bulgaricus* and L. *lactis*, L. *delbrueckii* is retained and the other two are designated as subspecies (Marshall, 1987).

Dietary fiber (DF), an extrinsic additive to milk, is known to have wide ranging beneficial effects on human health. The fortification of milk and milk products with DF may be the result of four main reasons, firstly from the considerable work done by various researchers/product developers in order to either enhance the fiber content of the product or replacement of fat for the obvious health benefits, secondly, to achieve some of the technological benefits e.g., improvement in the texture, thirdly, as cheap bulking agent along with artificial sweeteners or micronutrient premixes, and fourth reason may be the enhanced sensory benefits, as fiber rich dairy foods produced traditionally. Due to the growing double burden of diseases because of under nutrition on one hand and the over nutrition on the other, it is becoming essential to develop DF fortified milk and milk products on large scale. Such innovations in product development will probably help the people and the governments to address the problem of malnutrition and several metabolic diseases. Among the commercially available concentrated source of DF, psyllium husk is the most searched one primarily because of its solubility and functionality. Hence, all the yoghurt drinks designed were maintained acceptable viable counts i.e. L. *bulgaricus* and S. *thermophilus* with good probiotic activity.

According to Food Safety and Standards Authority of India (FSSAI), 2011 and codex standards, 2003, the viable count of S.*therophilus and* L.*bulgaricus*, standard value should be not less than 10^7 per gram. Counts of all the set yoghurts and yoghurt drinks remained in the acceptable range and thus these yoghurt samples had probiotic properties.

CONCLUSIONS

Addition of psyllium husk fiber in experimental yoghurts had showed good viability for probiotic organisms compared with both control samples. The research in the areas of food science and technology is expanding rapidly with new knowledge to develop or design new food product with enhanced viability of probiotic organisims. The designer yoghurts which are developed in current study had good probiotic properties. Hence the designer set yoghurt and yoghurt drink developed in the current study can scale up and suggested to commercialize by the functional food industry.

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