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Formulation of dairy based probiotic drink using the lactic acid bacteria of fermented cereals

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Abstract

Probiotics are considered as viable preparations in foods or dietary supplements to improve the health of humans and animals The aim of this research was to evaluate some probiotic traits of *Weissella confusa* and *Bifidobacterium bifidum* strains previously isolated from fermented cereals. Strains of *W. confusa* and *B. bifidum* were used to prepare probiotic drinks in single and in combination. The formulated probiotic drink was evaluated for the following properties such as pH, stability, and shelf life, before releasing them to market. When the data were satistically compared it was found that the corresponding P value of the pH level of test drink did not undergo any significant change. Whereas organoleptic analysis showed a variation among them in their consistency. The determination of Shelf Life showed that there was a notable reduction in the number of CFU after every 7days of storage, but the Chi-square test revealed that this reduction was also not significant. Thus the results clearly show that there was no marked reduction in the viability and pH of the dairy based probiotic drink and also it showed good shelf life.

Key words: Probiotic, yoghurt, supplements, stability, Shelf life, organoleptic

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INTRODUCTION

Probiotics were defined as microbial derived factors that stimulate the growth of other microorganisms. In 1989 Roy Fuller suggested a definition of Probiotics which states that '*A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance'* which has been widely used. Probiotics were thought to beneficially affect the host by improving its intestinal microbial balance, thus inhibiting pathogens and toxin producing bacteria (Metchnikoff, 1907). In the present day context specific health effects are being investigated and documented including alleviation of chronic intestinal inflammatory diseases (Mach , 2006), prevention and treatment of pathogen-induced diarrhea (Yan and Polk, 2006) urogenital infections, and atopic diseases. The ability of lactobacilli and bifidobacteria to survive in and colonize the gastrointestinal track has been associated with various health promoting properties. The colonization of those bacteria decreased with the increase of age of the host (Ballongue, 2004). In the recent years there has been interest in incorporating these bacteria in live form (called probiotics) into food especially fermented milk to counteract harmful bacteria in the gastrointestinal track and to promote health effect (Schillinger et al., 2005; Tamime et al., 2007). Several criteria have to be met with for selecting probiotic strains. Those include acid and bile tolerance, survival through the gastrointestinal track, ability to adhere to intestinal surfaces, exhibiting antimicrobial activity against potential pathogenic bacteria and good technological properties (Ouwehand et al., 2004).

In the present work strains of *Weissella confusa and Bifidobacterium bifidum* were isolated from various fermented cereals using Lactobacillus MRS agar and identified based on Bergy's manual, and the probiotic potentiality was evaluated. They were labeled as S2 (*Weissella confusa*) and S3 (*Bifidobacterium bifidum) and its combination was labeled as S2S3* (*Weissella confusa and Bifidobacterium bifidum*). The isolated organisms were used to prepare probiotic yoghurt.

MATERIALS AND METHODS

Formulation of the Product

Preparation of fermented milk product

For the production of fermented milk, skim milk base (containing 11% skim milk powder, 1% glucose and 0.2% yeast extract) was pasteurized at 90ÚC for 10 minutes prior to fermentation, cooled to 4ÚC and then divided into three parts. One portion was inoculated with 1% of S2 culture; another portion with S3 culture and the third portion was inoculated with combination of S2 and S3. Each inoculated mixer was incubated at 37ÚC until it reaches pH 4.5 and then cooled to 4ÚC (Xiao *et al.,* 2003). Thus three different fermented milk products (yoghurt) were prepared

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using the test organisms both individually and in combination.

Evaluation of the formulated product

The formulated products containing probiotic organisms should be evaluated for the following properties before releasing them, which include, stability, shelf life, impact on the consumer with regard to nutritional status, pathogenic microflora, immune status and lactose tolerance ability.

Stability

The stability of the product is governed by its pH, which may affect the organoleptic properties of the product. Hence the formulated probiotic product, which was stored at 4ºC, was analyzed for their pH using pH meter. The organoleptic properties which include characteristics like texture, consistency, flavor, etc., were also examined to check the quality of the product.

Shelf life

The shelf life of the product was determined once in a week for a period of one month by using pour plate technique and checking the viable cell count with commercial yoghurt as control. One gram of each yoghurt sample was diluted with 9ml of sterile peptone water. Subsequently serial dilutions were made and the samples were inoculated onto the MRS agar plates and then the plates were incubated at 37°C for 24 h. Plates were examined and colony forming units were recorded (Akalin *et al.,* 2004).

RESULTS AND DISCUSSION

Table.1. Changes in pH of yoghurt

Duration (in days)	S2 yoghurt	S3 yoghurt	S2+S3 yoghurt	Control
0	4.5	4.49	4.51	4.5
7	4.49	4.48	4.49	4.49
14	4.48	4.47	4.48	4.47
12	4.47	4.46	4.47	4.46
28	4.44	4.44	4.44	4.44

Table.1a. ANOVA pH

Groups	N	Mean	SD	SE	F Value	P Value
S2	5	4.48	0.02	0.01	0.184	0.906 (NS)
S3	5	4.47	0.02	0.07		
S2S3	5	4.48	0.03	0.01		
Control	5	4.47	0.03	0.02		
Total	20	4.47	0.02	0.05		

S2 – *Weissella confusa* , S3 – *Bifidobacterium bifidum,* NS – Non-significant

Table.2. Shelf life of Probiotic Bacteria in the product (Viability) CFU / gm

Product	Number of days				
	0	7	14	21	28
S2 yoghurt($x10^8$)	112	80	69	57	45
S3 yoghurt ($x10^8$)	104	76	61	52	39
S2S3yoghurt ($\times 10^8$)	128	96	82	65	40
	70+58	52+44	45+37	39+26	28+12
	(S2)(S3)	(S2)(S3)	(S2)(S3)	(S2)(S3)	(S2)(S3)
Control ($x10^8$)	123	78	64	55	42

S2 – *Weissella confusa* ,S3 – *Bifidobacterium bifidum* Overall Chi-square - 82.15, P value - 0.0000, Degrees of freedom 16

Formulation of the product

Consumption of non pathogenic, lactic acid – producing bacteria as part of the diet for their potential beneficial effects has been a dietary practice for centuries and has increased over the past decade. Fermented milk products are a common part of the diet in Asia, Europe and parts of Africa. Tuomola *et al.* (1998) stated that the dominant food vehicles for probiotics remain to be yoghurts and fermented milks, both of which provide a relatively low - pH environment in which the probiotic bacteria must survive.

So yoghurt was prepared by fermented milk using *Bifidobacterium* and *Weissella* both individually and in combination. Similarly, Saavedra *et al.* (2004) in their study used *Bifidobacteria* and *Lactobacilli* as isolated forms and in combination for the preparation of fermented milk. In the present study yoghurt was prepared using S2, S3 and S2S3 cultures, and by inoculating these cultures in to 11% skimmed milk powder. When the pH reached 4.5 the product was taken and stored at 4°C., and used for further analysis

Product evaluation

Stability

The stability of the probiotic yoghurt was checked, using parameters such as pH and organoleptic analysis. The stability of the prepared product was checked by determining the pH of the product when stored at 4ºC for the period of 1 month. pH ranged from 4.44 to 4.51 (Table 1), which indicated that pH of the product did not change during storage, and the product was stable. This was confirmed by ANOVA

(Table 1a). The ANOVA table was constructed for the variables S2, S3, S2S3 and control. From the corresponding P value it is inferred that the pH level does not undergo any significant change.

The initial pH values of the samples were ranged from 4.51 to 4.49. The pH of all samples decreased slightly during storage and did not drop below 4 at the end of storage. There was no major difference in pH values at 4°C for up to 28 days. This could probably be due to low acidifying activity of the yoghurt and probiotic cultures (Table 1). The drop in the pH was similar for all the yoghurt samples (Table 1a).

Shah *et al.* (1995) and Akalin *et al.* (2004) also found that there was a similar decrease in pH values during storage of commercial yoghurt containing *L. acidophilus* and *B. bifidum*, as well as in *B. longum* and *B. animalis* fermented milk. Similarly, the initial pH values in yoghurts containing *L. acidophilus* and *Bifidobacteria* decreased from 4.4-4.3 at day 0 to 4.2 at the end of 35 days of storage (Dave and Shah, 1997). Shah (2000) reported that the pH of the product was 4.5 or lower to meet legal requirements and to produce good quality yoghurt. It has also been reported that the concentrations of acetic acid could vary in the product depending on the extent of growth of probiotic bacteria. In the present study study it was found that the pH of the product did not change during storage and the product was stable.

Organoleptic analysis

Organoleptic analysis included the taste, texture, flavour and the consistency of the product. However both of them had a good flavour. Many different strains and dosage patterns for the production of Yoghurt have been reported in various studies. The yoghurt prepared using *Bifidobacterium* was white, thick hard in consistency without much whey and with an excellent flavour, whereas yoghurt produced using *Weissella* was pale white in colour, loose in consistency with whey, and the flavour was also acceptable .In case of combination of yoghurt, it was white, thick and soft in consistency similar to the Bifido yoghurt, and also with good flavour. This could be due to the factor that *Bifidobacterium*, is capable of controlling the whey. Fox *et al.* (1993) stated that the proteolytic and lipolytic properties determine the degradation of proteins and lipids, which have considerable effect on the taste and flavour of dairy products.

Shelf life

A comparative account of change in the viable count of probiotic bacteria in S2, S3 and S2S3 yoghurt during refrigerated storage is presented in Table 2. Shelf life of the product was determined once in a week for a period of one month, by using pour plate technique, where the viable cells were counted (Table 2). There was a notable variation in the viable count in yoghurts fermented with S2 and S3. The number of viable cells was more in S2 sample ($112x10^8$cfu) on 0 day where as it reduced gradually in the subsequent weeks and it was $45x10^8$cfu on the twenty eighth day. In the case of S3 yoghurt, the load of viable cells was less than the S2 sample ($104x10^8$ cfu) which reduced gradually to $39x10^8$ cfu whereas control showed an initial load of $123x10^8$ cfu which was lowered during further storage to about $42x10^8$ cfu. Chi-square test revealed that this reduction is not significant (Table 2a).

There was a gradual decrease in cfu from 0 day to 28 days in all the product samples. However the level of reduction was not significant enough to affect the product quality as evidenced by chi-square test (Table 2a).

In a similar study on *S. thermophilus* it has been reported that viable count was slightly reduced to around 10-15% during the storage of yoghurts containing *Bifidobacterium* (Medina and Jordano, 1994; Dave and Shah, 1997; Rybka and Fleet, 1997). Akalin *et al.* (2004) found that *Lactobacillus delbruecki* subsp. *bulgaricus* showed a marked decrease of 97-99% in their number when compared to

S. thermophilus during refrigerated storage. However the survival rate of

S. thermophilus was better than that of both *L. delbruecki* subsp *bulgaricus* and *Bifidobacteria.* These observations are in line with those of Kim *et al.* (1993), Medina and Jordano (1994), Lim *et al.* (1995) Dave and Shah (1997).

A number of brands of commercial yoghurts were analyzed in Australia and in Europe by Iwana *et al.* (1993) for *L. acidophilus* and *Bifidobacteria*. However certain reports showed that the viability of probiotic bacteria was affected by inhibitory substances such as lactic acid produced during production and cold storage. During fermentation these organisms produced organic acids but the concentration varied with the organisms (Shah, 2000).

The viability of probiotic bacteria in yoghurt depends on the strains used, interaction between species present, culture conditions, production of hydrogen peroxide due to bacterial metabolism, final acidity of the product and the concentrations of lactic and acetic acid. The viability also depends on the availability of nutrients, growth promoters and inhibitors, concentration of sugars, dissolved oxygen and oxygen permeation through package especially for *Bifidobacterium* spp inoculation levels, incubation temperature, fermentation time and storage temperature (Bertoni *et al.,* 1994).

The main factors for the loss of viability could be decrease in pH of the medium and accumulation of organic acids as a result of growth and fermentation (Shah and Jelen, 1990). *Bifidobacteria* are anaerobic in nature and hence higher oxygen content could affect the growth and viability. The availability of growth factors has also been reported to affect the growth and viability of probiotic bacteria (Shah, 2000).

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