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Research Paper

Fermentation dynamics of probiotic *Lactobacillus acidophilus* NCDC-13 in a composite dairy-cereal substrate

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ABSTRACT

The current investigation reports the search for a multipurpose composite substrate composed of dairy and cereal components for probiotic organisms. Probiotic *Lactobacillus acidophilus* NCDC 13 was inoculated in a substrate containing different combinations of whey-skim milk (WSM), germinated pearl millet flour (GPMF) and liquid barley malt extract (LBME). *L. acidophilus* NCDC 13 grew well in all the substrate combinations, though WSM + LBME was a better substrate than WSM + GPMF. Increasing levels of GPMF and LBME had a positive effect on the fermentation dynamics, acidification rate and the viability of probiotic culture. WSM in combination with 5% GPMF and 3% LBME resulted in the highest growth rate of the probiotic organism. The growth rate as well as acidification rate could be enhanced at low generation times by increasing inoculation levels to 4% and 5% and incubating for 8 and 6 h respectively. As the viable cell counts were above the required standards, 4.0% inoculum levels for 8 h fermentation time was selected as optimum. A base model for probiotic fermented formulations containing dairy and cereal ingredients with quick fermentation, high probiotic counts and a suitable pH range for acceptability of the fermented foods was established during the investigation.

Keywords: Lactobacillus acidophilus, *Probiotic, Fermentation dynamics, Pearl millet, barley, whey*

Introduction

Probiotics are live microorganisms that confer a beneficial effect on the host when administered in proper amounts (Brown and Valiere, 2004). Multiple reports describe the health benefits of probiotic organisms such as gastrointestinal infections, antimicrobial activity, improvement in lactose metabolism, reduction in serum cholesterol, immune system stimulation, antimutagenic, anti-carcinogenic and anti-diarrheal properties, improvement in inflammatory bowel disease and suppression of *Helicobacter pylori* infection by addition of selected strains to food products

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(Agerholm-Larsen *et al.*, 2000; Gotcheva *et al.*, 2002; Nomoto, 2005; Imasse *et al.*, 2007). The ability of any probiotic strain to attain and sustain a high cell population in a product is of primary importance. A concentration of approx. 10^8 cells ml^{-1} at the time of consumption is considered functional (Lourens-Hattingh and Viljeon, 2001). High cell growth and acidification rates would also result in the reduction of fermentation times and enhance the viability of the specific strain by preventing growth of undesirable micro-organisms present in the raw material (Marklinder and Lonner, 1992). The substrate composition and nutritional requirements of the probiotic strain considerably affect the overall fermentation process. Microbial growth also depends on environmental factors such as pH, temperature and accumulation of metabolic end-products.

Cereals are grown over 73% of the total world harvested area and comprise over 60% of the world food production (Charalampopoulos *et al.*, 2002b). They are the cheapest source of energy and supply large quantities of calorie and protein particularly in the developing countries. Cereals have higher content of some essential vitamins, dietary fibre and minerals compared to milk, but have lesser quantities of readily fermentable carbohydrates (Charalampopoulos *et al.*, 2002b). Lactic acid fermentation of cereals has been known since ancient times. Most of the cereal-based fermented foods are produced indigenously in Asia and Africa by natural uncontrolled lactic acid fermentation (Oyewole, 1997), that relies on native microbial population of the raw material resulting in products that exhibit substantial variations in flavour and quality (Giraud *et al.*, 1998). A potentially probiotic starter culture may ferment a cereal substrate and produce a fermented food with defined and consistent characteristics and possibly health-promoting properties. However, several technological aspects have to be considered in the design of such a novel food fermentation process, such as the composition and processing of the raw material, the growth capacity and productivity of the starter culture and the stability of the final product during storage (De Vuyst, 2000).

Whole grain is a good delivery vehicle for probiotics. A way to increase the nutritional value of cereal-based products is by combining cereals and milk. Milk, though a good source of nutrients lacks in fibre, whereas, cereals are rich source of fibre which increases the intestinal transit time of bowels, binds carcinogens and can provide several health beneficial effects. The proteins present in milk have high biological value and good amino acid profiles whereas cereal proteins have low digestibility and are deficient in certain amino acids.

Whey is the major by-product of Indian dairy industry, which is mainly discharged into the environment leading to significant loss of protein and energy source. The biochemical oxygen demand (BOD) of whey varies from 30,000 to 50,000 mg g^{-1} depending upon the milk solid contents in the whey (Mukhopadhyay *et al.*, 2003) and whey treatment as an effluent is very expensive. Alternatively, conversion of the liquid whey into beverage (i.e. fermented or not) is one of the most attractive options for the utilization of the whey for human consumption due to the excellent

functional and nutritional properties of the whey proteins (Gandhi and Patel, 1994).

India is among the leading producers of minor cereals and millets but many of these cereals such as barley and pearl millet are used merely as fodder crops despite their nutritive value. Their inclusion in the regular food chain would provide an economic incentive to farmers besides the obvious nutritional advantages. Exploiting the potentially synergistic qualities of whey and minor cereals - both under-utilised products - and harnessing them with the healthful attributes of probiotics has immense potential in developing healthful foods. The current study was aimed at investigating the fermentation pattern of a probiotic lactobacillus species in a composite dairy-cereal mix. The objective of the fermentation study was to develop a base model for fermented formulations containing dairy and cereal ingredients to achieve the following advantages: 1) quick fermentation to limit the risk of microbial contamination, 2) high viable cell concentration to ensure probiotic effect of the foods and 3) a suitable pH range for acceptability of the foods.

Materials and methods

Materials

A dairy isolate probiotic strain *Lactobacillus acidophilus* NCDC-13 was obtained from National Collection of Dairy Cultures (NCDC) at National Dairy Research Institute, Karnal (India). The freeze-dried cultures were activated in chalk litmus milk at 37°C for 24 h and grown in 100 ml of MRS broth at 37°C for 24-48 h. Subsequently, the organisms were grown in reconstituted skim-milk (10% w/v) with at least two sub-culturings, before they were used to prepare the 16 h old inoculum containing ~ 8×10^8 cfu ml^{-1} used in these experiments. Acid whey (*paneer* whey) (TS 6%, fat 0.25%, pH 5.5) was filtered and mixed with skim milk (TS 9%, fat 0.15%, pH 6.7) in the ratio 60:40 to obtain whey-skim milk (WSM). The pH of the mixture was adjusted to 6.8 with 25% KOH. Pearl millet grains purchased from the local market were cleaned, washed and soaked in water in the ratio 1:3 for 24 h at ambient temperature. The soak water was changed every 12 h. The soak water was drained and the seeds were left to germinate by incubating at 25-35°C for 48 h. The germinated grains were dried at 55-60°C for 8 h, ground and sieved through 52 mesh sieve to obtain germinated pearl millet flour (GPMF). Liquid barley malt extract (LBME) (solids by refractometer 80.5% , pH 5.72, protein on dry basis 5.17%, ash 1.453%, specific gravity 1.398) was obtained from Malt Company (India) Limited, Gurgaon, India. All other chemicals were purchased from standard manufacturers.

Preparation of optimised substrate

Different levels of GPMF (0, 1, 3, 5, 7% w/v) and LBME (0, 1, 2, 3% w/v) were added in WSM separately and sterilized in order to select the optimum levels of the cereals on the basis of pH and viable cell count. Once selected, these optimum levels of GPMF and LBME were added in WSM, the mixture heated at 95°C for 10

min and cooled to 37°C to make the optimised substrate (OS). The growth characteristics of *L. acidophilus* NCDC-13 (1% inoculum) were studied in OS at 37°C till death phase.

Enumeration of viable cells

Different levels (1, 3, 4, 5% v/v) of *L. acidophilus* NCDC 13 were inoculated in OS, resulting in average respective counts of 8×10^6, 2.4×10^7, 3.2×10^7 and 4×10^7 cfu.ml^{-1} and fermentation carried out at 37°C for 6-8 h. All enumeration of probiotic organism was done in salicin (0.5%) modified MRS medium (Dave and Shah, 1996) following the pour-plate method described by Houghtby *et al.* (1993).

Determination of pH, titratable acidity

The pH was determined using pH meter (Labindia, India). The titratable acidity in terms of percent lactic acid was determined by modifying the method described by AOAC (1995) methods for cheese. Five grams of sample was mixed homogenously by adding 20 ml hot distilled water (65°C), followed by addition of 10 ml of 0.1 N NaOH and one ml of 0.5% phenolphthalein indicator, before titrating against 0.1 N HCl.

Specific growth rate

The specific growth rate (k) for each substrate was calculated from the following equation:

$$k\ (h^{-1}) = 2.303(\log_{10}X_2 - \log_{10}X_1)/\ (t_2 - t_1)$$

where, X_1 and X_2 were cell counts at times t_1 and t_2 respectively (Cogan, 1978). The reciprocal of specific growth rate was recorded as generation time (Pelczar *et al.,* 2004).

Acidification rate

The change in pH during fermentation was monitored at 2 h intervals up to 8 h. The final acidification rate (V_f) was calculated as the variation of pH as a function of time (dpH/dt) and expressed as 10^{-3} pH units/min.

Statistical analysis

Data were recorded as mean±SE of three independent replicates and subjected to one-way analysis of variance (ANOVA) with a least significant difference of 95% using SPSS (version 16.0) software. Mean values were compared using the Tukey test at ($P < 0.05$), and different letters were used to label values with statistically significant differences among them.

Results and discussion

Effect of Germinated Pearl Millet Flour (GPMF) on fermentation dynamics

Figure 1A illustrates the effect of GPMF on pH and viable count. Increasing levels

of GPMF led to decrease in pH, which can be related with increasing number of probiotic counts. The specific growth rates (k) after 8 h at 0, 1, 3, 5 and 7% GPMF level were 0.617, 0.689, 0.808, 0.933 and 0.949 h^{-1} respectively (Table 1). There was an 11.69% increase in specific growth rate when 1% GPMF was added in WSM, probably due to the presence of fermentable nutrients in GPMF. The specific growth rate increased further to 30.84%, 51.45% and 53.73% respectively on addition of 3%, 5% and 7% of GPMF in WSM. Lactobacilli are known to require complex media containing numerous amino acids, vitamins and related growth factors in addition to fermentable carbohydrates (Morishita *et al.* 1981; Gomes and Malcata, 1999). The increase in growth rate due to addition of GPMF in milk substrate may be due to increase in availability of different nutrient contents such as different sugars or minerals such as manganese, iron, zinc etc., which is an important growth factor of organism (Blandino *et al.* 2003).

The acidification rate (V_f) varied from 1.125×10^{-3} to 2.805×10^{-3} pH units min^{-1} with significant differences between them. The highest value was observed with 7% GPMF, and the lowest, in control (no GPMF). Addition of 1% GPMF significantly enhanced acidification ability, which continued to increase with increment in GPMF content. Enhancement of acidification rate was earlier observed by several workers due to addition of casein hydrolysate (Oliveira *et al.*, 2001), inulin (Oliveira *et al.*, 2009) and milk protein hydrolysates (Lucas *et al*., 2004). Champagane *et al.* (2009) recorded faster acidification ability of three pure probiotic cultures in soy beverages than in milk. Significant effect on culture growth and viability was noted at 24 h incubation due to addition of rice fibre (Fernando *et al.*, 2011). Pea fibre, pea protein and lentil flour significantly ($P<0.05$) enhanced acidification rate in some yoghurt cultures. Milk supplemented with these pulse ingredients improved acidification rate of probiotic cultures, and highest effect was observed with lentil and soy flour (Zare *et al.*, 2012).

The generation time (t_g) reduced from 97.28 min in control to 64.34 min and 63.23 min in 5% and 7% GPMF respectively. A significant ($P<0.05$) reduction (10.42%) in generation time was observed with addition of 1% GPMF. Higher reduction was recorded (33.86% and 35%) with addition of 5% and 7% GPMF respectively in control, though they did not differ significantly. As the increase in specific growth rate as well as, generation time at 5% was not significantly different from that at 7% GPMF, 5% GPMF was selected as optimum for further studies.

Effect of Liquid Barley Malt Extract (LBME) on fermentation dynamics

Level of LBME as well as the fermentation time had highly significant (Pd"0.01) effect on pH change and viable cell count, as is evident from Figure 1B. The probiotic growth was higher in LBME than GPMF which could be due to presence of fermentable sugars like maltose, sucrose, glucose, fructose and free amino nitrogen (Charalampopoulos *et al.*, 2002a). Cell count increased with increasing levels of LBME, resulting in higher accumulation of lactic acid leading to decrease in pH

Table 1: Growth of *L. acidophilus* NCDC-13 in different substrates (1% inoculum)

Substrate	Level (% w/v)	k (h^{-1})	% Increase in k	V_f ($\times 10^{-3}$ pH units min^{-1})	t_g (min)	% Decrease in t_g
Control (Whey-Skim milk (60:40) (WSM)	-	0.617±0.008[a]		1.125±0.024[a]	97.28±1.3[a]	-
GPMF in WSM	1	0.689±0.006[b]	11.69	1.597±0.037[b]	87.14±0.73[b]	10.42
	3	0.808±0.004[c]	30.84	2.229±0.021[c]	74.26±0.33[c]	23.66
	5	0.933±0.005[d]	51.45	2.646±0.024[d]	64.34±0.34[d]	33.86
	7	0.949±0.005[d]	53.73	2.805±0.007[e]	63.23±0.24[d]	35.00
LBME in WSM	1	0.902±0.006[e]	46.19	2.472±0.028[f]	66.55±0.426[e]	31.62
	2	0.952±0.006[f]	54.29	2.917±0.012[g]	63.053±0.41[f]	35.22
	3	1.076±0.008[g]	74.39	3.125±0.012[h]	55.78±0.405[g]	42.69
Optimized substrate (GPMF & LBME in WSM)	5 & 3 resp.	1.23±0.024[h]	99.35	3.27±0.065[i]	48.95±0.83[h]	49.68

Values with different superscripted letter in a column differs significantly ($p < 0.05$).
k- specific growth rate, V_f - acidification rate, t_g - generation time

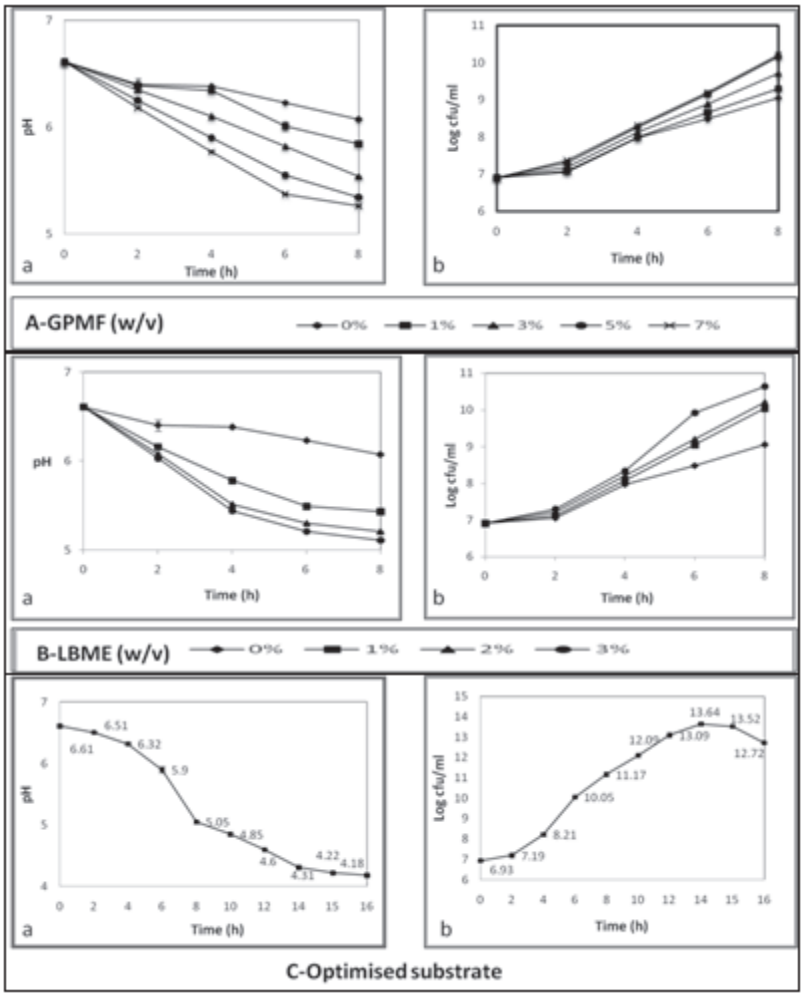

Figure 1: Growth characteristics of *L. acidophilus* NCDC-13 (inoculum level 8×10^8 cfu ml^{-1}) in (A) GPMF, (B) LBME, (C) Optimised substrate. Changes in (a) pH and (b) viable probiotic cell count

level. The lowest pH (5.11) was at 3% LBME with highest probiotic count (4×10^{10}). The specific growth rates (k) observed after 8 h of fermentation at 0, 1, 2 and 3% LBME were 0.617, 0.902, 0.952 and 1.076 h^{-1} respectively (Table 1). There was 46.19%, 54.29% and 74.39% increase respectively in specific growth rate due to addition of 1, 2, and 3% of LBME. Angelov *et al.* (2006) reported that increase in oat flour content from 4% to 5.5% led to increase of cell counts at 2.65 and 2.41 log orders, respectively. Incorporation of 1% LBME resulted in one log count increase in probiotic count in the current experiments. Charalampopoulos *et al.* (2002a) investigated overall growth kinetics of four potentially probiotic strains (*L.*

fermentum, L. reuteri, L. acidophilus and *L. plantarum*) in malt, barley and wheat media and observed that in the malt medium, all strains attained maximum high cell populations. The final acidification rate at 8 h varied from 1.125×10^{-3} pH units min^{-1} in control to 3.125×10^{-3} pH units min^{-1} in 3% LBME and was statistically significant ($P<0.05$). Addition of 3% LBME resulted in highest increase in acidification rate. Increment in acidification rate was concomitant with reduction in generation time. Addition of 1%, 2% and 3% LBME resulted in respective reduction of 31.62%, 35.22% and 42.69% in generation time, which is a measure of the effective growth of organism. As 3% LBME addition in WSM attained very high increase (74.39%) in specific growth rate, significant increase in acidification rate and considerable (42.69%) decrease in generation time, this level was selected as optimum for further study.

Comparison of different substrates on the fermentation dynamics

The organism grew well in all substrates (Table 1). This may be due to enrichment in nutrient content due to combination of dairy and cereal components. On the basis of specific growth rate which is an indicator of the ability of microorganisms to utilize the substrate (Fernando *et al.*, 2011), it is evident that LBME (k 1.076 h^{-1} @ 3% LBME) was a better substrate than GPMF (k 0.808 h^{-1} @ 3% GPMF). Addition of 3% GPMF resulted in 30.84% increase in specific growth rate from control, whereas corresponding increase due to 3% LBME was 74.39%. However, addition of 5% GPMF caused 51.45% increase in specific growth rate than control containing no cereals. Different growth and fermentation rates of probiotics as a function of carbohydrates have been recorded for *Lactobacillus* spp. (Chervaux *et al.*, 2000; Zhang and Lovitt, 2006) and *Bifidobacterium spp.* (Mlobeli *et al*., 1998; Perrin *et al.*, 2001). Charalampopoulos *et al.* (2002a) also reported maximum growth rate in malt medium for four probiotic organisms.

In the current study, the optimised substrate (WSM + 5% GPMF+ 3% LBME) supported the growth to the maximum level. In the optimised substrate the specific growth rate was 1.23 h^{-1} (generation time 48.95 min), which resulted in increment in specific growth rate to 99% than control. This result may be attributed to combination of the two cereal substrates (GPMF and LBME at optimum levels) in milk and resulting high enrichment of nutrients that supported the growth of the organism. Generation time has been suggested by a few investigators as an important tool for investigation of the microbial dynamics either in pure or mixed cultures (Bruno *et al.*, 2002; Liong and Shah, 2005). The generation time at standard condition for *L. acidophilus* in milk at 37°C lie between 66-87 min (Pelczar *et al.*, 2004). In the current study, in WSM which did not contain any cereal, the generation time was 97.28 min. The longer generation time may be attributed to deficiency of certain nutrients in WSM and different fermentation conditions. The generation time reduced to 74.40 min and 64.34 min due to addition of 3% and 5% GPMF respectively and further to 55.78 min on addition of 3% LBME and was lowest (48.95 min) in the optimised substrate. Helland *et al.* (2004) studied the growth and metabolism of

four probiotic strains in milk- and water-based puddings containing both maize and rice flour. All strains showed good growth and survival in milk-based puddings (8–9.1 log cfu g^{-1}). Significantly lower cell counts were obtained during fermentation and storage of water-based puddings, indicating both lower growth and stability of the probiotic cultures in these products.

Growth Characteristics of NCDC-13 in optimised substrate

Based on the above results the optimised substrate (OS) was prepared with WSM containing 5% GPMF and 3% LBME. An exponential growth phase of 12-13 h was observed for NCDC-13 in the OS with 1% inoculum. Stationary phase was reached after 14 h of incubation, when cell densities remained almost constant (13.65±0.02 $\log_{10}$cfu ml^{-1} and 13.52±0.04 $\log_{10}$cfu ml^{-1}) during stationary phase (Figure 1C). However, the cell population (12.72±0.04 $\log_{10}$ cfu ml^{-1}) declined highly significantly (Pd"0.01) after the end of stationary phase. Since all fermentations were performed under no pH control, the organic acids formed via the metabolic pathways may have decreased the pH of the media. The population densities and pH recorded at the end of exponential phase was 13.1±0.05 $\log_{10}$ cfu ml^{-1} and 4.5-4.6 respectively. At the end of the stationary phase, pH values dropped from 6.61±0.006 to 4.22±0.0145, whereas, the acidity increased from 0.12±0.006 to 1.03±0.014% LA. The specific growth rate (k) of *L. acidophilus* in optimised substrate was 1.23 h^{-1} (equivalent to a generation time of 48.95 min).

Charalampopoulos *et al.* (2002a) observed an exponential growth phase of 6-8 h for *L. fermentum* and *L. acidophilus*, while *L. reuteri* and *L. plantarum* grew exponentially until 10–12 h of fermentation. They reported that the cell population of *L. acidophilus* significantly declined after the end of the exponential phase, whereas viable cell densities of *L. fermentum, L. plantarum* and *L. reuteri* declined slightly during the stationary phase. All fermentation resulted in decrease in pH. Rozada-Sanchez *et al.* (2008) monitored the growth kinetics of four *Bifidobacterium* sp. in malt hydrolysate and observed that the counts of all organisms increased exponentially between 0 and 10 h and became approximately constant after that. Fernando *et al.* (2011) reported a very short stationary phase and long exponential phase due to fermentation of rice fibre by combination of different genus of lactobacilli and bifidobacteria, which indicated a greater utilization of fibre by microorganisms. He observed a maximum cell count of 10^{10}-10^{12} cfu/ml after 24 h fermentation time. When probiotics are added to fermented foods, one of the important factors that influences the survival and activity of the product during the gastrointestinal transit is the physiological state of the probiotic organisms in probiotic foods. They should be present in food in the logarithmic or the stationary growth phase (Rivera-Espinoza and Gallardo-Navarro, 2010). Attempts were made to maintain fermentation in exponential growth phase with viable cell count in the range of 12-13 $\log_{10}$ cfu $ml^{-1}$and pH of the substrate in the range of 4.0-4.5 so that they lie in the acceptable range.

Effect of starter culture concentration on fermentation dynamics

The optimised substrate was inoculated with 1, 3, 4 and 5% starter culture of *L. acidophilus* (NCDC-13) in order to achieve the high levels of viable counts of probiotic organisms in short fermentation times. Short fermentation times lead to improvement in plant output, quick preparation of product, reduced cost of electricity as well as lower number of unwanted contaminating microorganisms (Angelov *et al*., 2006). Furthermore, cultures can generate metabolites with unpleasant flavours in long fermentation times (Macedo *et al*., 1998). Results of the fermentations are presented in Figure 2. The growth rate was enhanced by increasing inoculation levels. The desired pure range of 4.51 and 4.6 with adequate viable count (13.22 and 13 $\log_{10}$ cfu ml^{-1} respectively) was obtained in significantly shorter times of 8 and 6 h respectively, with 4% and 5% inoculum compared to 1%, which took 12 h to reach pH 4.6 with corresponding probiotic count of 13 $\log_{10}$ cfu ml^{-1}. The acid production rate was also monitored (Table 2) as it affects the time required for processing which is critical to economics (Zare *et al.*, 2012). The final acidification rate increased from 3.27×10^{-3} pH units min^{-1} to 3.49×10^{-3} pH units min^{-1}, 4.36×10^{-3} pH units min^{-1} and 4.94×10^{-3} pH units min^{-1} due to application of 1%, 3%, 4% and 5% inoculum levels respectively. Increasing inoculum levels also resulted in reduction

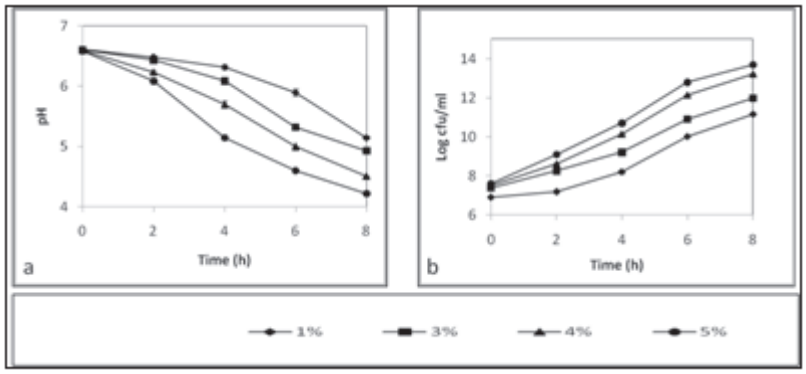

Figure 2: Effect of culture concentration on fermentation dynamics of optimised substrate. Changes in (a) pH and (b) viable probiotic cell count

Table 2: Effect of different culture concentrations on the growth of the organism in optimized substrate

Culture level (% v/v)	k (h^{-1})	V_f ($\times10^{-3}$ pH units min^{-1})	t_g (min)
1 .	1.23±0.021[a]	3.27±0.065[a]	48.95±0.83[a]
3 .	1.33±0.003[b]	3.49±0.077[b]	45.28±0.09[b]
4 .	1.65±0.007[c]	4.36±0.019[c]	36.38±0.16[c]
5.	1.76±0.017[d]	4.94±0.032[d]	34.19±0.32[d]

Values with different superscripted letter in a column differs significantly ($p < 0.05$).
k- specific growth rate, V_f - acidification rate, t_g - generation time

in generation time from 48.95 min (1%) to 45.289 min (3%), 36.38 min (4%) and 34.19 min (5%). The corresponding values for specific growth rate were 1.23, 1.33, 1.65 and 1.76 h^{-1} respectively. Reduction in generation time contributed to quick fermentation. Application of 4% and 5% inoculum resulted in about 26% and 30% reduction in generation time as compared to 1% inoulum level.

High cell growth and acidification rates helps to reduce fermentation time and boost the viability of specific strains in fermentation medium (Markinder and Lonner, 1992) and presumably in human gut (Fernando *et al.*, 2011). Angelov *et al.* (2006) also reduced fermentation time to 8 h with pH level 4-4.5 when fermenting a whole-grain oat substrate with probiotic strain *L. plantarum* B28 with 5% inoculum level to obtain a drink with viable counts 9.3×10^9 cfu ml^{-1}. Similarly Marklinder and Lonner (1992) achieved pH 4.3-4.5 in 6 h when fermenting oatmeal soups with *L. plantarum* strains, while Martensson *et al.* (2002) reported pH 3.9-4.5 after much longer fermentation (16 h) of an oat base with commercial mixed dairy cultures. The different fermentation rates could be attributed both to strain specificities as well as differences in substrate. As the cell counts achieved in 6 h and 8 h with both 5% and 4% levels of inoculum were above the required standards, it was more prudent to use 4% inoculum levels with 8 h fermentation time.

Conclusion

The current study established a base model for fermented formulations containing dairy and cereal ingredients with the following attributes: 1) Substrate - WSM (60:40) + GPMF (5% w/v) + LBME (3% w/v), 2) Inoculum level - 4% v/v, 3) Fermentation time – 8 h, 4) pH of product - 4.4-4.6 and 5) Probiotic count – 10^{12} to 10^{13}cfu ml^{-1}. Thus, the aim to achieve quick fermentation to limit the risk of microbial contamination, high viable counts of probiotic organisms and a suitable pH range for acceptability of the foods was established. This model can be applied in multiple probiotic food products like probiotic beverages (sweet or spicy), porridges, gruels and set and/or stirred yoghurt-like products. These products would be cost-effective owing to inexpensive raw material. Their functional attributes may prove an economical option for the management of diarrhoea and malnutrition in underprivileged populations, at the same time providing a new market option for the health food sector.

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