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Original Research Article

Control of Diarrhoea by Probiotic Fermented Food Blend

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A B S T R A C T

A food mixture containing dehusked barley flour, green gram dhal flour, skimmed milk powder and fresh tomato pulp (2:1:1:1, w/w) was developed. It was developed, cooled and fermented with *L. acidophilus* (10^5 cells/ml) at 37^0C for 24 hours. Probiotic fermentation reduced the contents of starch, phytic acid and polyphenols, improved the digestibility of starch and protein and enhance the availability of sodium and potassium. Such a fermented mixture exhibited antibacterial activity towards pathogens namely *E.coli, S.dysenteriae* and Salmonella typhosa. Feeding of fermented BGMT drink reduced the faecal coliforms and increased the faecal lactobacilli counts in children suffering from diarrhoea leading to complete relief from diarrhoea in 7 out of 10 children.

Introduction

Diarrhoeal disease, one of the leading causes of morbidity and mortality among infants and young children in developing countries, is estimated to kill five million children worldwide per year (Guerrant *et al.*, 1990).

Diarrhoea can cause under nutrition and worsen milder forms of malnutrition due to faecal losses. Moreover, food withholding by parents or health professionals who believe that children should not be fed during the acute phase of diarrhoea may also contribute to the decline in nutritional status.

Although some malabsorption of macronutrients does occur during acute diarrhoea, yet 80 to 95 percent of carbohydrate, 70 percent of fat and 75 percent of nitrogen are actually absorbed from mixed diets based on common foods.

Recent studies conducted by WHO revealed that infants receiving total colorie requirement from the first day of therapy for diarrhoea absorbed more nutrients, retained more nitrogen and gained more weight as compared with infants receiving half the recommended calories, Hence, alternatives to the classical glucose based oral rehydration solution, primarily formulations based on rice, barley, other cereals and legumes are also being investigated.

No doubt, homemade solutions based on careals, legumes of their blends will have a better quality protein, good profile of carbohydrates and minelas but will contain considerable amount of antinutritional factors (Ganguli *et al.*, 2014) which limit their utilization. Fermentation of such a cereal-legume blend with L. Acidophilus, a

probiotic organism may not only bring some desirable nutritional changes but when ingested along with the food, may also change the microbial composition in the intestinal tract through competitive exclusion or production of antibiotics and hence, control diarrhoea. Keeping it in view, the present study was planned to develop probiotic fermented food mixture, study its nutritional composition and role in control of diarrhoea in children.

Materials and Methods

Materials

Huskless barley and green gram dhal (phaseohis aureus) were produced from the deptt. Of plant breeding, Haryana Agricultural University, Hisar, India. Skimmed milk powder was collected from National Dairy Research Institute, Karnal, India, whereas tomatoes were purchased from the local market in a singal lot. Seed less tomato pulp was obtained by mashing and sieving the blanched tomatoes in a thick strainer.

Barley and green gram dhal were cleaned and ground in an electric grinder (Cemotec 1090, M/s Tecator, Hgonas, Sweden) using 1.5 mm sieve.

Microbial Cultures

Pure culture of *Lactobacillus acidophilus* strain R employed for fermentedtion was collected from National collection of Microorganisms unit, NDRI, Karnal, India and propagated routinely in sterile skimmed milk.

Three test organisms namely *Shigella dysenteriae*, *Salmonella typhosa* and *Escherichia* coli were also procured from the above mentaioned source.

Development of Fermented Indigenous Food blend

The developed food blend i.e. BGMT contained freshly ground huskless barley flour, green gram dhal flour, skimmed milk powder and tomato pulp in 2:1:1:1 (w/w) proportion. The developed food blend (100 g) was mixed with distilled water (600 ml), stirred sufficiently to obtain a homogenous slurry, autoclave (121ºC, 15 min), cooled, inoculated with pure culture of *L. acidophilus* (10^5 cells/ml) and fermented at 37ºC for 24 hrs. Fermentation was carried out in triplicate. The unfermented blend slurries before and after autoclaving served as controls.

Determination of Antibacterial Activity

Antibacterial activity of fermented and unfermented food blend was determined by Agar Well Assay technique as recommended by British Standard Method (British Standards, 1974)

Chemical Analysis

Titratable acidity was determined as lactic acid per 100 ml by the method of Amerine *et al.,* (1967), whereas pH was measured by a pH meter against a standard buffer of 4.0.

Curd protein, ash, NDF and ADF contents were analysed using standard methods (AOAC, 1990). Total soluble sugars were estimated by the method of Yemm and Willis (1954) and starch from the sugar free pellet was estimated by the method of Clegg (1956). *In vitro* starch digestibility was assessed by employing pancreatic amylase (Singh *et al.*, 1982). Protein digestibility (*in vitro*) was carried out by the method of Akeson and Stahmann (1964) as modified by Singh and Jambunathan (1981). Phytic acid was determined in the sample extracted

with 0.2 N HCL for 3 hrs. Using bipyridine solution (Haug and Lantzsch, 1983). HCL extractability of sodium and potassium i.e. an index of their bioavailability was determined by flame photometer.

Feeding Trial

Ten children in the age group of 1-3 years who were suffering from diarrhoea for the last 2-3 days were selected from labour colony, HAU Campus, Hisar, India. These children were poor and could not afford to take medicines. Therefore, they were not taking antibiotics or any other kind of medical treatment for diarrhoea.

One hundred gram of fermented BGMT slurry was mixed with cooled sterilized water (100 ml) and salt (1.5%) to mask the sourness of the product and fed to each child as a buttermilk drink for six days. The subjects took their usual meals during the period under study and no restriction was made regarding the type of foods to be consumed. The faecal samples of children were collected prior to feeding of fermented drink, on 2^{nd} and 6^{th} day of the feeding trial. The faecal samples were analysed for coliforms and lactobacilli counts.

The data were subjected to analysis of variance in a completely randomised design.

Results and Discussion

Titratable Acidity and pH

The pH of unfermented BGMT mixture i.e. 5.38 dropeed to 3.30 after fermentation. Tiratable acidity (2.56 to 3.02 g lactic acid/100ml) rose simultaneously with a decline in pH of fermented BGMT mixture. A significant ($P>0.01$) negative correlation (-0.9275) was noticed between pH and titratable acidity. The homofermentative *L. acidophilus* converts glucose to lactic acid which is responsible for the decline in pH of the product.

Antibacterial Activity

The diameters of methanol acetone cell free extract inhibition zones of fermented BGMT mixture towards pathogenic organism viz. *Shigella dysenteriae*, *Salmonella typhosa* and *Escherichia coli* were 19, 16 and 24 mm, respectively.

Hence, growth of all the pathogenic organisms was inhibited by cell free extract obtained from fermented BGMT mixture whereas unfermented mixture had no inhibitory zone.

The production of lactic acid and ensuing reduction in pH, production of H_2O_2 and some natural antibiotic substances namely acidophilin, acidolin, lactobacillin and lactocidin by *L. acidophilus* (Khedkar et.al. 1990) may be responsible for inhibitory effect of cell free extract of fermented BGMT mixture towards pathogenic organisms.

Nutrient Composition

Raw unprocessed BGMT mixture contained protein, ash, NDF and ADF as 18.6, 2.41, 2.44 and 0.63 g/100 g, respectively (Table - 1). Due to autoclaving, no significant change occurred in the contents of these nutrients. However, protein content decreased significantly ($P<0.05$) as a result of fermentation which may be attributed to an increase in protein catabolism by the fermenting microorganisms which lead to the escaping of the byproduct of metabolic deamination i.e. ammonia. These findings are similar to those reported in pearl millet fermented with fermentum or *L. Brevis* (Khetarpaul and Chauhan, 1989)

Since no addition or deletion of mineral source was involved during autoclaving or fermentation, ash content remained unaltered during these processing methods. NDF and ADF contents increased in the autoclaved BGMT Mixture which further decreased during fermentation but these changes were not significant. Reduction in soluble fibre on autoclaving (Hughes and Swanson, 1989) might have resulted in slight increased in NDF and ADF, the insoluble dietary fibres. The reduction in NDF and ADF contents after fermentation may be due to solubilisation of fibre. Taguchi *et al.,* (1986) also observed that total dietary fibre in natto and tempeh decreased slightly during fermentation. Total soluble sugars increased, whereas starch content was decreased when BGMT mixture was subjected to autoclaving. After fermentation, total soluble sugars as well as starch content were lowered down significantly (Table 1). Increase in the level of soluble sugars upon autoclaving may be due to degradation of starch and formation of simple sugars. As a result of fermentation, a significant decrease in the starch content can be attributed to amylolytic action of microbes causing the breakdown of starch into soluble sugars. Fermenting microbes have been reported to contain both beta and alpha amylases (Bernfeld, 1962). Which increase in the period of fermentation, these sugars may also be utilized by the fermenting microflora and therefore, the fermented product may exhibit sugar levels lower than that observed at the initial stage of fermentation. Similar findings have been observed earlier in pearl millet (Khetapaul and Chauhan, 1991).

Unprocessed BGMT mixture had 220.22 mg phytic acid per 100 g which lowered down significantly during autoclaving and fermentation (Table 1). Fermented mixture had 63 percent less phytic acid over the control value. A wide range of microflora have been known to process phytase activity (Lopez *et al.,* 1983) which may partly be responsible for reduction in phytic acid content.

The *in vitro* digestibility of starch (expressed as mg maltose released per g sample) and protein (%) of unprocessed BGMT mixture was 31.55 and 50.99, respectively (Table 1). Starch digestibility almost doubled in the fermented product. Protein digestibility inproved to the extent of 51 percent over the control value. Enhanced digestibility of cooked cereal and legume starches by alpha amylase could be attributed to swelling and rupturing of starch granules which facilitates more randomized configuration for L.amylase to affect amylolytic hydrolysis and inhibition of L.amylase inhibitors (Subbulakshmi *et al.,* 1976). The presence of alpha amylase in the fermenting bacteria brings about cleavage of amylose and amylopectin to approximately six parts of maltose and five parts of glucose and hence, improvement occurs in starch digestibility. Improvement in protein digestibility of fermented product is mainly associated with enhanced proteolytic activity of fermenting microflora (Sindhu and Khetarpaul, 2003). High proteinase activity has been reported in fermented foods (Steinkraus, 1983).

Phytic acid known to inhibit proteolysis (Knuckles *et al.,* 1985) and amylolysis (Thompson and Yoon, 1984) is considerably reduced during fermentation (Table 1) which may also partly explain the improvement in starch and protein digestibility. A significant and negative correlation was found between phytic acid and digestibility (*In vitro*). The diminishing effect of fermentation on polyphenols level may be due to the activity of polyphenol oxidase present in the food grains or microflora (Sindhu and Khetarpaul, 2001).

Table.1 Nutrient composition of *L. acidophilus* fermented BGMT* food blend

Nutrients	Processing treatments			CD
	Raw blend (Control)	Unfermented autoclaved mixture	Fermented autoclaved mixture	(P<0.05)
Crude protein (g/100 g)	18.6 ± 0.38	18.5 ± 0.37	17 ± 0.31	0.47
Ash (g/100 g)	2.41 ± 0.03	2.42 ± 0.02	2.42 ± 0.02	0.36
Neutral detergent fibre (g100 g)	2.44 ± 0.08	3.17 ± 0.07	2.85 ± 0.10	0.84
Acid detergent fibre (g/100 g)	0.63 ± 0.06	1.22 ± 0.08	0.85 ± 0.03	0.63
Total soluble sugar (g/100 g)	1.68 ± 0.03	4.33 ± 0.07	2.15 ± 0.04	0.10
starch (g/100 g)	37.6 ± 0.76	31.1 ± 0.95	18.5 ± 0.72	2.21
Polyphenols (mg/100 g)	614.6 ± 13.2	530.0 ± 12.2	484.4 ± 16.2	18.8
Phytic acid (mg/ 100 g)	220.2 ± 6.89	163.6 ± 5.49	81.5 ± 3.65	8.48
In vitro starch digestibility (mg maltose released/ g meal)	31.6 ± 2.76	53.0 ± 4.68	64.0 ± 3.43	4.49
In vitro protein digestibility (%) HCI-extractability (%)	51.0 ± 1.99	56.2 ± 1.67	76.8 ± 1.79	2.04
Sodium	61.4 ± 0.3	65.4 ± 0.6	73.6 ± 0.2	0.92
Potassium	61.9 ± 0.6	66.0 ± 0.3	70.5 ± 0.7	1.56

Values are means ± SD of three independent determinations.

*BGMT mixture contains dehusked barley flour, green gram dhal flour, fresh tomato pulp and skimmed milk powder (2:1:1:1, w/w).

Table.2 Effect of feeding fermented mixture containing barley flour, green gram flour, skimmed milk powder and tomato pulp on faecal lactobacilli counts of children (values are expressed as log viable counts per gram of the faeces)

Children designated as	Before feeding	During feeding	
		2 days	6 days
A_1	8.91 ± 0.06	9.10 ± 0.03	10.34 ± 0.01
A_2	7.18 ± 0.02	7.26 ± 0.01	8.78 ± 0.04
A_3	8.27 ± 0.02	8.38 ± 0.01	9.20 ± 0.02
A_4	7.10 ± 0.04	7.16 ± 0.02	7.98 ± 0.04
A_5	8.44 ± 0.01	8.65 ± 0.08	9.43 ± 0.01
A_6	7.07 ± 0.03	7.21 ± 0.02	7.36 ± 0.02
A_7	7.58 ± 0.07	7.83 ± 0.04	9.18 ± 0.04
A_8	8.72 ± 0.07	8.97 ± 0.04	9.62 ± 0.08
A_9	7.43 ± 0.01	7.59 ± 0.07	8.45 ± 0.02
A_{10}	8.20 ± 0.02	8.30 ± 0.02	9.65 ± 0.07
Average	7.89 ± 0.66	8.04 ± 0.69	8.99 ± 0.68
SE (m)	± 0.15		
CD (P<0.05)	0.32		

Children were given 100 g of fermented mixture containing *L. acidophilus*- R 2 x 10^9 cfu/g orally.

Table.3 Effect of feeding fermented mixture containing barley flour, green gram flour, skimmed milk powder and tomato pulp on faecal coliform counts of children (values are expressed as log viable counts per gram of the faeces)

Children designated as	Before feeding	During feeding	
		2 days	6 days
A_1	9.60 ± 0.04	9.56 ± 0.05	8.96 ± 0.03
A_2	10.03 ± 0.03	9.91 ± 0.04	9.13 ± 0.02
A_3	9.55 ± 0.05	9.33 ± 0.02	9.09 ± 0.02
A_4	9.41 ± 0.02	9.35 ± 0.02	8.79 ± 0.04
A_5	9.44 ± 0.02	9.29 ± 0.05	8.25 ± 0.02
A_6	10.20 ± 0.03	10.05 ± 0.02	9.23 ± 0.01
A_7	9.59 ± 0.09	9.24 ± 0.02	8.70 ± 0.05
A_8	9.18 ± 0.02	9.04 ± 0.02	8.28 ± 0.082
A_9	9.80 ± 0.04	9.56± 0.06	8.99 ± 0.03
A_{10}	9.09 ± 0.02	8.90 ± 0.05	8.18 ± 0.03
Average	9.59 ± 0.33	9.42 ± 0.34	8.75 ± 0.37
SE (m)	± 0.15		
CD (P<0.05)	0.32		

Children were given 100 g of fermented mixture containing *L. acidophilus* – R 2 x 10^9 cfu/g orally.

HCI-extractability of sodium and potassium, an index of their bioavailability (Kim and Zemel, 1986) changed significantly due to fermentation. About 20 and 14 percent increase occurred in the extractability of sodium and potassium of fermented BGMT mixture, respectively when compared to unfermented BGMT mixture. Fermentation has been reported to enhance the extractability of minerals in fermented corn meal (Chompreeda and Fields, 1984) and cereal-legume blends (Goyal and Kheterpaul, 1994).

Feeding study

Upon feeding of fermented BGMT drink for six days to children suffering from diarrhoea, it was observed that 70 percent of the subjects completely recovered from diarrhoea. Twenty percent of the subjects felt some improvement, whereas 10 percent did not get relief. The faecal lactobacilli counts during diarrhoea ranged from 7.07 to 8.91 log viable cells/g among the 10 subjects and this level increased slightly after two days of feeding (Table 2). On 6th day of feeding trial, faecal lactobacilli counts increased significantly in nine subjects.

Faecal coliforms varied from 9.09 to 10.20 log viable counts/g in children suffering from diarrhoea. From the 2nd day onward after feeding fermented BGMT drink, the number of faecal coliforms started declining. At the end of experiment i.e. after six days, a significant (P<0.05) a reduction was noticed in the faecal cliform counts in the subjects individually as well on average basis (Table 3).

The reduction in faecal coliforms may be attributed to antibacterial activity of *L. acidophilus* fed along with the fermented BGMT drink, as also observed by rise in faecal lactobacilli counts on the 2nd and 6th day of feeding indicating its implantation in the GIT. *L. acidophilus* has been reported to reduce the intestinal *E. Coli* by competitive exclusion (Savage, 1983) and also by producing antimicrobial substance or a metabolite to neutralize the enterotoxin from *E. Coli* (Shahani *et al.,* 1977). Similar findings have also been reported by Prajapati *et al.,*

(1986) who fed a preparation containing *L. acidophilus*, banana paste, tomato juice concentrate and sugar with 200 ml pasteurized milk to adults for 15 days. They noticed that faecal lactobacilli counts increased from 41 to 550 million/g and faecal coliforms decreased from 7 million/g and faecal coliforms indicating successful implantation of *L. acidophilus.*

In conclusion, use of *L. acidophilus*-R, a probiotic organism should be encouraged in the preparation of fermented products not only to increase their nutritional value but also to check diarrhoea.

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