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EFFECT OF PROBIOTIC FERMENTATION ON THE *INVITRO* DIGESTIBILITY OF STARCH AND PROTEIN OF BANANA BASED FOOD MIXTURES

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Abstract

The consumers over whelming interest for functional foods, including probiotics have resulted in attempts to develop fourteen probiotic food mixtures containing banana flour, green gram flour, soya flour, tomato, mango and papaya. 25 g of each mixture was mixed with 150 ml of distilled water and adjusted the pH to 4.5 and autoclaved at 121° C (1.5 kg/cm^2) for 15 mts. After cooling this was inoculated with 300μl (119×10^6 cfu/ml) liquid culture of *L.acidophilus* (24 hour old culture) and incubated at 37° C for 24 hours. After fermentation it was freeze dried and the samples were analysed for thier organoleptic qualities, viability of *L acidophilus*, *invitro* starch (IVSD) and *invitro* protein (IVPD) digestibility. The unfermented samples served as control. The IVSD and IVPD varied between 78.57% to 83.60 % and 85.14% to 86.21% respectively in fermented food mixtures. The viability of *L. acidophilis* varied from 136 to 292 x 10^{7}cfu/g.

Keywords: Probiotic, *L. acidophilus*, *invitro* digestibility, organoleptic qualities

1. INTRODUCTION

Probiotic foods are those foods which contain a live microbiological culture either as a result of fermentation or as an intentional addition to beneficially affect the host by improving the intestinal microbial balance [1].

Traditional probiotic foods are acid fermented dairy products, such as yoghurt [2]. Most probiotic foods are fermented at least partially and the products which have received the most attention in this regard include fermented milks, such as yoghurt and buttermilk, as well as unfermented milks with cultures added [3,4,5], frozen desserts such as ice cream and frozen yoghurt [6], miso, kefir, sauerkraut, certain pickles, tofu, tempeh etc.

Probiotic food constitutes a sizeable part of the functional food market [7], and continues to grow at an exponential rate, with the potential for market growth estimated at a staggering US$ 120 million per month [8]. After many years of popularity in the Japanese and European markets, manufacturers of these products are venturing into new markets, including the Arabian Gulf region, as evidenced by the variety of probiotic food products now available in supermarkets and health food stores [9]. A staple based food mixture if developed from commonly used foods in the community and then fermented with probiotic organism, may have a better profile of nutrients, acceptability and therapeutic value. Therefore, in the present study, an attempt has been made to develop a banana based probiotic fermented food mixtures and to report the sensory qualities, viability of *L. acidophilus* and the *invitro* digestibility of starch and protein of the developed food mixtures.

2. MATERIALS AND METHODS

2.1. Collection of raw materials and preparation of food mixtures

Raw banana (Nendran *Musa* AAB) was purchased from the local market. This was peeled, washed, sliced and dried. The dried chips were powdered to a flour of 40 mesh size. This banana flour was used as a source of starch in all food mixtures. The foods selected for developing the probiotically fermented food mixtures were defatted soya flour and green gram flour (as source of protein in the food mixture), mango, papaya and tomato and these foods were purchased from the local market.

In the present study, *L.acidophilus* was used as the probiotic culture for the fermentation of food mixtures. Pure cultures of *L.acidophilus* (MTCC 447) used was obtained from Institute of Microbial Technology (IMTECH), Chandigarh

2.2. Development of food mixtures

The food mixture was fermented under optimum conditions with a control.

2.2.1. Autoclaved and fermented food mixture (FFM): The food mixture (25g) was mixed with 150ml water and stirred to obtain uniform slurry. Adjusted the pH to 4.5 and autoclaved at 121° C (1.5 kg/cm^2) for 15 mts. After cooling this was inoculated with 300µl(119×10^6 cfu/ml) liquid culture of *L.acidophilus* (24 hour old culture) and incubated at 37° C for 24 hours. After fermentation it was freeze dried.

2.2.2. Autoclaved and unfermented food Mixture (UFFM): The food mixture (25g) was mixed with 150ml water and stirred to obtain uniform slurry. Adjusted the pH to 4.5 and autoclaved at 121° C (1.5 kg/cm2) for 15 mts. After cooling it was freeze dried.

2.3. Organoleptic qualities of the food mixtures

A series of acceptability trials were carried out using simple triangle test at the laboratory level and selected a panel of ten judges between the age group of 18-35 years as suggested by [10].

Sensory evaluation of the developed food mixtures were carried out in the morning using score cards based on a five point hedonic scale by a panel of 10 selected judges. Sensory evaluation of the developed food mixtures were conducted by mixing 5g of the food mixture in 100 ml of diluted buttermilk(1:4) and was done in the morning using score cards based on 9 point hedonic scale by the selected panel of 10 judges. The quality attributes namely appearance, colour, flavour, texture, taste and overall acceptability were evaluated.

2.4. *invitro* digestibility of starch and protein

Starch digestibility was estimated as suggested by Standard procedure [11]. Later glucose was estimated by the method of [12].IVPD was determined by the method [13].

2.5. Viable count of *L.acidophilus*

Viable counts of *L.acidophilus* present in fermented food mixture were enumerated using MRS medium. One gram of the mixture was weighed and transferred to a tube containing 9ml sterile distilled water (dilution 10^{-1}). This was then serially diluted upto 10^{-7}.The samples were enumerated for microbial count by pour plate method using MRS agar and the results are expressed as cfu/g

3. RESULTS AND DISCUSSION

The foods selected for developing the probiotically fermented food mixtures were banana flour, defatted soya flour, green gram flour ripe mango, papaya and tomato. Fourteen food mixtures with various combinations were prepared and presented in Table1. All the food mixtures contained 60-70 percent banana as the major constituent and 20 percent of either defatted soya flour or green gram flour. Fruit pulps viz mango, papaya and tomato either singly or in combination were present in 10 – 20 per cent levels.

3.1. Organoleptic qualities of the food mixtures

Probiotic fermented food mixtures (5gm) mixed with 100ml of diluted buttermilk (1:4) were subjected to sensory evaluation .The corresponding control of unfermented samples were also presented in the same way. Mean scores obtained for both FFM and UFFM for different quality criteria were calculated and presented in Table 2.

As revealed in the table, the mean score for appearance, colour and texture of the fermented samples were liked very much (between 8-9 in hedonic scale) where as flavour, taste and over all acceptability were moderately liked (between 7-8 hedonic scale) by the panelists.

The benefits of fermentation may include improvement in palatability and acceptability by developing improved flavours and textures. Lactic acid fermentation enhances considerably the sensory properties of food resulting in a variety of tastes [14]. During fermentation, lactic acid bacteria, yeast and other bacteria contribute significantly to flavour development [15]. The cultures used in food fermentation are, however, also contributing secondary reactions to the formation of good flavour and texture [16].
As shown in table 3, the mean score for appearance, colour and texture of UFFM were liked very much (between 8-9 in hedonic scale)whereas flavour and taste were neither liked nor disliked (between 5and 6 in hedonic scale) by the panelists.

Statistical analysis by applying independent t test it was revealed that (table 4) there was no significant difference between the appearance, colour and texture of fermented and unfermented food mixtures but fermented mixture had significantly ($p<0.05$) higher acceptability scores with regard to flavour, taste and overall acceptability.

During fermentation, several volatile compounds are formed, which contribute to a complex blend of flavours in products. The presence of aromas represented by diacetyl, acetic acid and butyric acid makes fermented cereal-based products more appetizing [17]. The proteolytic activity of fermentation microorganisms often in combination with malt enzymes may produce precursors of flavour compounds, such as amino acids, which may be deaminated or decarboxylated to aldehydes and these may be oxidized to acids or reduced to alcohols [18]. However, the end product distribution of lactic acid fermentation depends also on the chemical composition of the substrate (carbohydrate content, presence of electron acceptors, nitrogen availability) and the environmental conditions (pH, temperature, aerobiosis/ anaerobiosis), controlling of which would allow specific fermentations to be channelled towards a more desirable product .

A probiotic fermented food made from pearl millet flour , chick pea flour , skim milk powder and fresh tomato pulp (2:1:1:1) with *L. acidophilus* (10^5 cells/ml) at 37 °C for 25 h showed good acceptability [19].

The BCGT (Barley flour, milk coprecipitate, sprouted green gram paste and tomato pulp 2:1:1:1 w/w) probiotic food mixture developed by [20] was found acceptable and the overall acceptability ranged from 'like slightly' to 'like very much'. A probiotic beverage developed based on cheese whey and soy with good sensory properties [20]. The organoleptic qualities of acidophilus yoghurt fermented with *L.acidophilus* 301 and normal yoghurt and found no significant differences in the textural characteristics and both the products were almost identical with respect to colour, flavour, appearance, texture and overall acceptability with a score ranging from 7.4 to 7.8 [21].

.3.2. IVSD in fermented and unfermented food mixtures

Table 5 shows the IVSD in FFM and UFFM. Among FFM, IVSD was maximum in T_4 (83.60 per cent) and minimum in T_2 (78.57 per cent). There was no significant difference in IVSD of T_4 with that of T_{10} (83.4 per cent) and T_{11} (83.37 per cent). Among UFFM, maximum IVSD was in T_9 (56.34 per cent) and least IVSD was in T_{12} (54.41 per cent).

3.3. IVPD in fermented and unfermented food mixtures

Table 6 shows the IVPD in FFM and UFFM. There was a significant variation in the IVPD of FFM and UFFM. IVPD of FFM varied from 85.14 to 86.21 per cent, the maximum in T4. T_4 showed no significant variation with T_{10} (86.18 per cent) and T_{11} (86.19 per cent).Among UFFM, maximum IVPD was in T_6 (57.87 per cent) and the least in T_1 and T_8 (57.15 per cent)

FFM and UFFM were statistically compared for their IVSD and IVPD by applying independent sample‘t’ test and is presented in table 7.

Table 7 revealed IVSD and IVPD in FFM were significantly high when compared to UFFM. Fermentation of food is an important method which significantly lowers the content of antinutrients and thereby improves the nutritive value of foods. Fermentation encourages the multiplication of selected organisms and their metabolic activities in food. If fermentation is carried out with probiotic organisms, it might have specific added advantages apart from improvement of nutritive value. In addition to nutrient synthesis, probiotic may improve the digestibility of some dietary nutrients such as carbohydrates, proteins and fats.

In the present study also fermentation of food mixture with lactic acid bacteria has been shown to increase the digestibility of starch and protein.The *in vitro* starch digestibility of unfermented food mixtures ranged between 54.41 to 56.34 per cent and this improved significantly upon fermentation to 78.57 to 83.60 per cent. Similar findings were reported by [23] where the starch digestibility of unfermented autoclaved RSMT mixture was 62.65 per cent which on fermentation improved to 78.33 per cent. It was reported that probiotic fermentation of indigenous food mixtures containing tomato pulp using *L. casie* and *L. plantarum* showed an improvement of the digestibilities of starch and protein [24].

The increase in starch digestibility of fermented products may be related to enzymatic properties of microbes, which ferment the substrate. The fermenting micro flora brings about the breakdown of starch to oligosaccarides. The enzymes bring about the cleavage of amylose and amylopectin to maltose and glucose. The presence of α amylase in the fermenting bacteria was noticed [25, 26]. Complete elimination of alpha –amylase inhibitors in most fermentation also contributes to improved starch digestibility.

The IVPD in the unfermented food mixtures ranged between, 57.15 to 57.87 percent which significantly increased on fermentation to 85.41 to 86.21 per cent. A significant difference between the protein digestibility of fermented and unfermented food mixtures was noted [23]. An increase in protein digestibility was also observed [27] in sorghum-green gram blend after fermentation.Enhanced protein digestibility after fermentation has been reported in cereal-legume-whey blends [28].

Indigenously developed RWGT food mixture which contained rice flour, whey, sprouted green gram paste and tomato pulp (2:1:1:1 w/w) fermented with 2% liquid culture (containing 10^6 cells/ml broth of *L. casei* and *L. plantarum*) showed a drastic reduction in the contents of phytic acid, polyphenols and trypsin inhibitor activity while significantly improving the in vitro digestibilities of starch and protein. Sequential culture fermentations brought about higher changes as compared to single culture fermentations [29]. Food mixture which contained barley flour, milk coprecipitate, sprouted green gram paste and tomato pulp and fermented with *S. boulardi* and *L. casei* resulted in maximum increase in starch digestibility by 96 per cent [24] and protein digestibility by 50 per cent.

The improvement in protein digestibility is mainly associated with the enhanced metabolic activity of fermenting oraganism [30]. An improvement in protein digestibility of fermented products is mainly associated with an enhanced proteolytic activity of the fermenting microflora. High proteinase activity has been reported by various workers in fermented protein [31]. The improvement in IVPD caused by fermentation could be attributed to the partial degradation of complex storage proteins to more simple and soluble products [27] it could also be attributed to the degradation of tannins, polyphenols and phytic acid by microbial enzymes. The increase in digestibility may also be due to reduced antinutrient content of the fermented foods as antinutrients are known to inhibit amylosis and proteolytic activity [32]. An increase in amino nitrogen by fermentation signifies partial breakdown of protein to peptide and amino acid, resulting in improved protein digestibility [33, 34]

3.4 . Viable count of *L.acidophilus* in fermented and unfermented food mixtures

L. acidophilus present in FFM were enumerated and the results are presented in table 8.

As revealed in table 8, maximum viable count was observed in T_6 (292 cfu /g(x 10^7) and the least in T_{13} (136 cfu /g(x 10^7). As expressed in log cfu/g, the viable count of *L.acidophilus* in the treatments varied from 9.13 to 9.46 log cfu/g (Fig 9)as against the desired level of 4.7 to 8.9 log cfu/g in probiotic foods.

Viability and activity of the probiotic bacteria are important considerations, because the bacteria must survive in the food during shelf life and during transit through the acidic conditions of the stomach, and resist degradation by hydrolytic enzymes and bile salts in the small intestine [35].

To realize health benefits, probiotic bacteria must be viable and available at a high concentration, typically 10^6 cfu/g of product [36]. Products sold with any health claims should meet the criterion of a minimum 10^6 cfu/ml probiotic bacteria at the expiry date, because the minimum therapeutic dose per day is suggested to be 10^8–10^9 cells [37].

In the present study the maximum viable count was observed in T_6 (292 x 10^7 cfu/g) and least in T_{13} (136 x 10^7 cfu/g).The viability of *L .acidophilus* in the treatments varied from 9.13 to 9.46 log cfu/g as against the desirable level of 4.7 to 8.9 log cfu/g in probiotic foods [38].

Similar results were obtained by [24] in their BCGT (barley flour, milk coprecipitate, green gram and tomato pulp) food mixture. Single cell fermentation with *L.casei* resulted in a viable count of 9.88 log cfu/g and with *L. plantarum* showed a viable count of 9.11 cfu/g. A slurry using neutrallised acidophilus milk, banana paste, tomato juice concentrate and ground sugar at the rate of 40, 10 and 15 per cent respectively and observed a *Lactobacilli* count of 8.71 x 10^7 cfu/g [39].

Another study was by [40] who developed a new oat based probiotic drink fermented with lactic acid bacteria. He also observed a viable count of 9.3 x10^9 cfu/ml with 5 per cent inoculum concentration. Two indigenous food mixtures by mixing raw and germinated pearl millet flour, whey powder and tomato pulp fermenting with *L. acidophilus* and found that the growth of *L. acidophoilus* was significantly higher (8.64 cfu/g) in germinated flour mixture[41]. Suitability of tomato juice as a raw material for the production of probiotic juice by lactic acid bacteria and observed a viable cell count of 10^8 cfu/ml after fermentation of 72 h at 30°C [42]. Probiotic value of peanut flour fermented with different strain of lactic acid bacteria and found *L. plantarum* P9 grew to the highest cell population (9.48 log cfu/g) in peanut flour after 72h fermentation at 37^{o}C [43]. A probiotic oat based cereal bar fermented with *B.lactis* Bb-12 and found a viable count of 5x10^{9} cfu/ bar (25g) [44].

Cell viability in probiotic foods depends on the strains used, interaction between species present, culture condition, oxygen content, final acidity of the product and the concentration of lactic acid and acetic acid in the food system. Bacterial viability is important because many clinical studies suggest that live bacteria are mandatory to the beneficial effect of probiotic dietary supplements. In the present study all the 14 food mixtures showed a good viability of *L. acidophilus*.

4. Conclusion

In the present study, the mean score for overall acceptability of fermented products varied between 7.9 to 8.0 in a 9 point hedonic scale by a panel of 10 semi trained judges. The *invitro* digestibility of starch (82.10 per cent) and protein (85.85 per cent) was also significantly high in fermented food mixtures. Total viable count of *L acidophilus* ranged from 9.13 to 9.45 log cfu/g.

Fermentation has been used for centuries as means of improving the keeping quality of foods. Microorganism by virtue of their metabolic activities, contribute to the development of sensory, shelf life and nutritional qualities. Factors related to the technological and sensory aspects of probiotic food production are of utmost importance since only by satisfying the demands of the consumers can the food industry succeed in promoting the consumption of functional probiotic products.

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Table 1. Food combinations in the fourteen food mixtures

Food mixtures (Treatments)	Combinations (percent)
T_1	B-70, DS-20, M-10
T_2	B-60, DS-20, P-20
T_3	B-60, DS-20, T-20
T_4	B-70, GG-20, M-10
T_5	B-70, GG-20, P-10
T_6	B-60, GG-20, T-20
T_7	B-60, DS-20, M-10, P-10
T_8	B-60, DS-20, M-10, T-10
T_9	B-70, DS-,20, P-5, T-5
T_{10}	B-60, GG-20, M-10, P-10
T_{11}	B-70, GG-20, M-5, T-5
T_{12}	B-60, GG-20, P-10, T-10
T_{13}	B-70, DS-20, M-3.34, P-3.34, T-3.34
T_{14}	B-70, GG-20, M-3.34, P-3.34, T-3.34

B- Banana, DS- Defatted soya flour, GG- Green gram flour, M- Mango, T-Tomato, P-Papaya

Table 2. Mean score for organoleptic qualities of food mixtures fermented with *L. acidophilus*

Treatments	Mean score					
	Appearance	Colour	Flavour	Texture	Taste	Overall Acceptability
T_1	8.3	8.5	7.1	8.2	7.3	7.9
T_2	8.4	8.4	7.3	8.4	7.3	8.0
T_3	8.3	8.6	7.1	8.3	7.5	7.9
T_4	8.3	8.4	7.0	8.3	7.7	7.9
T_5	8.2	8.5	6.8	8.3	7.6	7.9
T_6	8.3	8.5	7.2	8.3	7.8	8.0
T_7	8.2	8.6	7.0	8.4	7.3	7.9
T_8	8.4	8.4	7.0	8.3	7.4	7.9
T_9	8.4	8.5	7.2	8.3	7.4	7.9
T_{10}	8.2	8.5	7.2	8.2	7.5	7.9
T_{11}	8.4	8.5	7.0	8.3	7.7	8.0
T_{12}	8.2	8.6	7.1	8.2	7.6	7.9
T_{13}	8.3	8.5	7.3	8.2	7.4	7.9
T_{14}	8.3	8.5	7.3	8.2	7.7	8.0

Table 3. Mean score for organoleptic qualities of unfermented food mixture

Treatments	Mean score					
	Appearance	Colour	Flavour	Texture	Taste	Overall Acceptability
T_1	8.3	8.4	5.6	8.3	5.5	7.2
T_2	8.3	8.4	5.8	8.2	5.6	7.3
T_3	8.2	8.5	5.8	8.2	5.7	7.3
T_4	8.2	8.6	5.7	8.2	5.7	7.3
T_5	8.3	8.5	5.6	8.2	5.7	7.3
T_6	8.3	8.5	5.6	8.2	5.6	7.2
T_7	8.3	8.6	5.8	8.2	5.6	7.3
T_8	8.4	8.4	5.5	8.4	5.8	7.3
T_9	8.3	8.5	5.8	8.2	5.5	7.3
T_{10}	8.3	8.4	5.8	8.3	5.8	7.3
T_{11}	8.4	8.4	5.5	8.3	5.8	7.3
T_{12}	8.3	8.6	5.6	8.3	5.6	7.3
T_{13}	8.3	8.5	5.7	8.4	5.8	7.3
T_{14}	8.4	8.5	5.7	8.3	5.8	7.3

Table 4. Comparison of Sensory qualities of fermented and unfermented food mixtures

Methods	Appearance	Colour	Flavour	Texture	Taste	OAA
Fermented	8.300	8.486	7.110	8.271	7.510	7.825
Unfermented	8.307	8.485	5.680	8.271	5.680	7.690
Mean difference	0.007	0.001	1.430	0.000	1.830	0.135
t value	0.041	0.044	21.35	0.039	26.34	7.154
Significance	NS	NS	S	NS	S	S

Values are mean of ten panelists

Table 5. IVSD in fermented and unfermented food mixtures

Treatments	IVSD (%)	
	FFM	UFFM
T_1	80.17 ^{g}	56.17 ^{f}
T_2	78.57 ^{h}	54.46 ^{g}
T_3	80.50 ^{g}	56.02 ^{e}
T_4	83.60 ^{a}	56.21 ^{b}
T_5	82.93 ^{cd}	56.23 ^{b}
T_6	83.07 ^{bc}	56.17 ^{c}
T_7	81.50 ^{f}	54.46 ^{g}
T_8	82.47 ^{e}	56.05 ^{e}
T_9	82.73 ^{cde}	56.34 ^{a}
T_{10}	83.40 ^{ab}	54.32 ^{j}
T_{11}	83.37 ^{ab}	54.36 i
T_{12}	82.90 ^{cd}	54.41 ^{h}
T_{13}	81.73 ^{f}	56.11 ^{d}
T_{14}	82.60 ^{de}	54.43 ^{gh}

Values are mean of three independent determinations

Values with same superscript do not have significant difference

DMRT column wise comparison

Table 6. IVPD in fermented and unfermented food mixtures

Treatments	IVPD (%)	
	FFM	UFFM
T_1	85.14 [g]	57.15 [h]
T_2	85.83 [d]	57.78 [c]
T_3	85.56 [f]	57.23 [g]
T_4	86.21 [a]	57.82 [b]
T_5	86.11 [c]	57.65 [d]
T_6	86.15 [b]	57.87 [a]
T_7	85.74 [e]	57.56 [e]
T_8	85.15 [g]	57.15 [h]
T_9	85.55 [f]	57.45 [f]
T_{10}	86.18 [ab]	57.65 [c]
T_{11}	86.19 [ab]	57.61 [c]
T_{12}	86.16 [b]	57.64 [c]
T_{13}	85.74 [e]	57.42 [f]
T_{14}	86.17 [b]	57.67 [d]

Values are mean of three independent determinations

Values with same superscript do not have significant difference

DMRT column wise comparison

Table 7. IVSD and IVPD in FFM and UFFM

Methods	IVSD (per cent)	IVPD(per cent)
FFM	82.109	85.850
UFFM	55.340	57.540
Mean difference	26.76	28.30
t value	103.49	416.57
Significance	.0001	.0001
	S	S

Table 8. Viable cell count of *L. acidophilus* in fermented food mixtures

Treatment	Viable count	
	cfu/g(x 10^7)	log cfu /g
UFFM	Nil	Nil
FFM		
T_1	147	9.167
T_2	139	9.143
T_3	282	9.451
T_4	159	9.201
T_5	143	9.155
T_6	292	9.465
T_7	137	9.136

T_8	210	9.322
T_9	205	9.311
T_{10}	147	9.167
T_{11}	188	9.276
T_{12}	175	9.245
T_{13}	136	9.136
T_{14}	275	9.439

Values are mean of 3 independent enumerations