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RESEARCH PAPER

Development and evaluation of probioticated food mixes

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Abstract

Great interest has recently been focused on the development of cereal based probiotic foods, since comparatively less work is available on cereal based probiotics. An attempt was made to develop such kind of probiotic food mixture which can be utilized to prepare different kind of food products which suits the Indian palate. Probiotic food mixes were developed using locally available ingredients such as Italian millet flour, wheat flour, soya flour, skimmed milk powder, roasted bengal gram dal powder in the proportion 30:40:11:3:15. Probiotic cultures i.e., single probiotic organism *Sporolacto bacillus* and mixed culture with the combination of *Streptococcus faecalis*, *Clostridium butyricum*, *Bacillus mesentericus* and *Lactobacillus* organisms were dehydrated using different dehydration techniques. Freeze drying provided higher survival rate of probiotic organisms i.e., 7.46×10^7 log CFU and 6.16×10^7 log CFU mixed probiotic cultures over spray drying. Moisture, protein, fat, crude fibre, carbohydrate, energy, ash, calcium, phosphorus, iron and zinc content of the developed composite probiotic functional mix were 11 g, 18.10 g, 5.70 g, 3.47 g, 58.77 g, 359 Kcal, 3.19 g, 107.10 mg, 343.22 mg, 5.66 mg and 2.03 mg per 100 g, respectively. Tannin, phytic acid, polyphenol and *in-vitro* protein digestibly were 111.50 mg, 3.1 mg, 120 mg and 55.3 per cent /100 g in the mix.Nutrient and non nutrient contents were the same in all the three mixes, since the ingredients used were the same except for probiotic culture powders. pH decreased and acidity percentage increased over a period of 60 days storage period in the mixes developed.

Keywords: Probiotics, Italian millet, spray drying, freeze drying

Consumers today are highly conscious of nutritionally rich, functional and therapeutic foods. Considerable interest has recently been focused on the development of probiotic and protein enriched fermented dairy products. The idea of using microbes to promote good health and to prevent disease is a method of tackling through natural and biotic applications to daily food. Initially, several microbes have been used unintentionally in food production such as dairy products and fermented vegetables. Such fermented foods are popular due to their distinct characteristic in terms of taste and aroma. In recent years, there has been a renewed interest in use of cultures microbial due to, apart from improving food flavour, their beneficial aspect in health restoration and disease treatment. Several microorganisms, under the name of "probiotics", have been proposed and used in a wide range of clinical trials, ranging from diarrheal disease to cancer prevention (Fuller, 1994; Kaur *et al.*, 2001).

The term "probiotics" was originally used by Lilley and Stillwell (1965) to mean a substance that stimulates growth of other microorganisms in the human intestine.

Most of the probiotic foods available at the market worldwide are milk based and very few attempts have been made for development of probiotic foods using cereals and legumes. Also there is very less literature available on cereal based probiotic products. If a staple based food mixture is developed from the commonly used cereals and legumes and then, fermented with a probiotic micro organism, it may have a better profile of nutrients and the therapeutic value. Most of the probiotics are selected from the group of lactic acid bacteria of which the lactobacilli species including *L.acidophilus, L.bifidus, L.casei and L.fermentum* are the most important ones. *L.acidophilus* is of major importance as it establishes an intimate relationship with man throughout life, starting from the time of birth.

Selecting a food matrix, which can carry a probiotic culture at effective levels, is a challenge. Most probiotic products are dairy-based since ancient times. Dairy based products provide of the a very nutritious substrate, which satisfies the nutritional requirements of fastidious LAB. But, expanding trend of vegan lifestyles, the issues of lactose intolerance, and the demand for low-fat and low-cholesterol foods have created a growing demand for non-dairy probiotic products. Consequently, whole cereals, millets which contain prebiotic constituents in the bran,are getting more attention as potential substrates. While granola bars and oat containing beverages are available in the market, many other cereals such as wheat, barley and malt have also been tested experimentally as food matrices for probiotics by tracking the viability of the inoculated probiotics strains (Charalampopoulos *et al.*, 2002).

The addition of skim milk powder to the cereal-legume blends can add nutrients as well as provide an optimum medium for the growth of probiotic organisms. But no such studies have been conducted till today, on the development of probiotic products based on cereal, legumes or their blends.

Dehydration of cultures

In view of keeping the cells intact for their health-imparting effects upon administration to human beings, freeze drying and spray drying methods have been prevalent during their commercial production. In freeze drying method, the first step of drying, cells are frozen and dried by sublimation under high vacuum using leak- proof vials. The freeze drying method provides higher survival of cells as compared to that of spray drying method, due to lower temperature and mild shock (if any) to the microbial cells.

On the contrary, spray drying method involves injection of cell mass fortified in spray drying medium and its exposure is controlled at i) higher velocity of hot air, ii) inlet and outlet temperature and iii) under mild vacuum for a short duration. Consequently water removal can lead to de-stabilization of the structural integrity of cellular components, resulting in a loss or impairment of designated functions. The primary target area of de-stabilization during drying is reported to be a lipid fraction of the cell membrane being affected due to peroxidation. This may result in reduced efficiency of DNA replication, transcription and translation and in turn, live character, as also health- imparting effect.

In order to achieve optimum results after spray drying of probiotics, attention must be focused on minimizing the damage to the cellular components. In this regard various researchers have noted that calcium in milk provides protective coating on bacterial cell wall and thereby increases their survival after dehydration.

Functional foods serve nutrients and physiologically active components for a healthy living. Composite flours are a mixture of flours from tubers rich in starch and/or protein rich flours (e.g. soy, peanut) and /or cereals (e.g. maize, wheat, rice, millet, buck wheat), with or without wheat flour (Seibel, 2001). Whole grains and millets were used as a base for developing these three composite mixes. This is because whole grains and millets are low cost nutritious and locally available foods. Whole grains are known to affect glucose and insulin responses, partly due to their slower rate of digestion as reported by Hallifrisch and Behall (2000). Whole grains and millets are also good sources of dietary fibre which helps to reduce the cholesterol levels in the body.

In the view of popularity of probiotic and its therapeutic use, probiotic food products were formulated from Italian millet which will enhance the availability of nutrients. Taking into consideration of nutritional and therapeutic values of probiotics an attempt has been made to develop probioticated food mixes.

Single culture probiotic as well as mixed culture of probioticorganisms were compared to know the effectiveness of probiotic organisms in millet based food preparations.

Materials and Methods

Italian millet (*Setariaitalica*) was procured from the Millet Scheme, UAS, GKVK, Bangalore other raw materials *viz.*, wheat flour (*Triticumaestivum*), defatted soya flour (*Glycine max*), skimmed milk powder, roasted bengal gram dal (*Cicerarietinum*), required to make composite food mix, were procured from the local super market.

Sporolac containing 50 million spores of *Sporolacto bacillus* and ViBact containing *Streptococcus faecalis*-30 million, *Clostridium butyricum*-2 million, *Bacillus mesentericus*-1 million and *Lactobacillus*-50 million probiotic dehydrated cultures were purchased from the medical shop and used as starter cultures.

Dehydration of the cultures

Culture (1 ml/ liter) was inoculated into the sterilized milk and kept under incubation for 24 hrs at 30°C. Calcium lactate was added to the culture at the rate of 20 per cent level as a protectant. Fermented milk was subjected to different dehydration techniques *viz.*, Spray drying of single culture and mixed culture were accomplished under optimized conditions (air inlet temperature 160°C, air pressure 2.5 kg/cm^2, flow rate @ 25 mL/min, Air outlet temperature 90 ± 1°C) and freeze dried for 34 hrs at -33.8° C using Lyodel lab model Lyophilizer.

The ingredients *viz.*, Italian millet flour, wheat flour, soya flour, skimmed milk powder, roasted bengal gram dal powder were mixed in the proportion 30:40:11:3:15 and were autoclaved. Lyophilized probiotic cultures were added at the rate of 1 g/100 g of the composite mix which contained 10^7 CFU probiotics. Three variations were made among the composite mixes *viz.*, Composite mix (control)- CM, Composite mix containing single probiotic culture- CMSP, Composite mix containing mixed culture-CMMP. The product made and was analysed for various physiochemical and nutritional characteristics.

Chemical analysis

Composite mixes were analyzed for macro and micronutrients and non-nutrients such as polyphenols, tannins and phytates. Analysis was done using standard methods (AOAC,1980; AOAC,1995) procedures. Carbohydrate content of the sample was computed by difference method. *Invitro* Protein Digestibility (IVPD) was analyzed according to Singh and Jambunathan (1982).

The pH was measured by using Elico digital pH meter against standard buffer of 4.0 and 7.0 pH. The acidity of the samples was determined by following the method of AOAC (1990).

Results and Discussion

The count of *Lactic acid bacteria* culture *i.e.*, single probiotic culture and mixed probiotic cultures before dehydration were 7.96 × 10^7 and 6.76 × 10^7 respectively. After spray drying count single culture was reduced to 0.59 × 10^7 whereas mixed culture reduced to 0.54 × 10^7. Freeze drying showed less reduction count of in probitoic organisms *i.e*. 7.46 × 10^7 and 6.16 × 10^7 respectively for both single and mixed cultures (Table 1). The recovery per cent was more in freeze drying (35.2) compared to spray drying (21.5) method. From these results, it is evident that freeze drying is better compared to spray drying with respect to survival rate as well as recovery. Meng *et al*. (2008) employed different drying techniques in drying of weaning foods. They opined that both freeze drying and spray drying can be used for manufacturing of probiotic powders on a large scale. Study conducted by Yadav *et al.* (2009) also showed similar results for viability of spray drying of probiotic organisms. The scientist found that calcium lactate as a best protectant minimizes the cellular loss in probiotic micro cultures.

Table 1: Effect of dehydration on bacterial population

Dehydration techniques	Population (CFU × 10^7/g)		
	Single culture	Mixed culture	Recovery %
Before dehydration	7.96	6.76	—
Spray drying	0.59	0.54	21.5
Freeze drying	7.46	6.16	35.2

Macro and micro nutrients

The prepared products were analysed for macro and micro nutritionals and the results obtained are summarized here.

The values obtained for moisture, protein, fat, crude fibre, carbohydrate, energy and ash of composite functional mix were 11.00 g, 18.10 g, 5.70 g, 3.47 g, 59.00 g, 359 Kcal and 3.19 g, respectively. Micro nutrient content in the mix were: calcium-107.10 mg, phosphrous-343.22 mg, iron-5.66 mg and zinc-2.03 mg (Table 2). Gupta and Sehgal (1991) also developed composite mixes by using pearl millet or barley with roasted amaranth, green gram and jaggery in the ratio of 60:20:40:45. The developed mixes contained 5.90 g moisture, 9.84 g to 9.95 g protein, 416 to 441 Kcal energy, 3.77 to 4.32 g ash, 17.75 to 19.42 mg iron and 150 to 190 mg calcium per 100g, respectively. Barbecue snacks prepared from composite mix by Prabhavat *et al.* (2000) also showed 24.10 to 26.39 protein, 23.59 to 26.49 fat and crude fiber 1.69 to 2.47. Nutritional characteristics of marama-sorghum composite flours and porridges were investigated by Kayitesi (2009). Compositing sorghum meal with marama flour significantly increased protein (61 to 96) and fat (5.6 to 14.6) contents in flours and porridges compared to sorghum porridge. Energy value and Lysine content also increased in marama composite flour. Grain based composite mixes developed by Teradal (2013) also showed 15.2 g moisture, 18.3g protein, 5.2 g fat, 5.9 g crude fibre, 48.3 g carbohydrate, 313 Kcal energy and 6.8 g ash per 100 gram of the sample.

Table 2: Nutrient composition of the composite mix/100g on dry weight basis

Nutrients	Composition
Proximate (g)	
Moisture	11.00
Protein	18.10
Fat	5.70
Ash	3.19
Fibre	3.47
Carbohydrates	59.00
Energy (Kcal)	359
Minerals (mg)	
Calcium	107.10
Phosphorus	343.22
Iron	5.66
Zinc	2.03
Anti-nutrients (mg)	
Phytic acid	3.10
Tannin	111.50
Polyphenols	120.00
***In Vitro* Protein Digestibility**	
IVPD	55.30%

Fat is a concentrated source of energy and increases the energy density of the diet. Fat in the dietalso helps in the absorption of beta carotene and other fat soluble vitamins (Gopalan *et al.*, 2004). Emphasis should therefore be placed on reducing the intake of saturated fat and choosing mono unsaturated fat sources. In the present study, overall higher values of fat may be attributed to the addition of functional ingredients soybean and Italian millet.

Higher fibre content as well as fat in the present study can be attributed to the *in situ* composition of the grain. Millets are known for their fibre content. In fact the fibre in mixes was less compared to that in the grain due to the processing operation employed *i.e.*, passing through 60 mesh sieve with a higher recovery of bran. Elevated levels of fat and carbohydrates might have also contributed to the increased energy value of Italian millet based mixes (Sahu, 1987).

In earlier study also emphasis is placed on complex carbohydrates and controlling the simple sugars. Whole grain cereals, millets and pulses were included in the composite mixes. According to Raghuram *et al.* (1997) 60-70 of the total calories should be contributed by the carbohydrates. In the present study, carbohydrate content was 58.77 g/100g. The marginally lower carbohydrates in these mixes have an advantage for diabetic and hypocholestrolemic diet.

A composite mix should provide more than 360 Kcal of energy as per PAG recommendation (Anon, 1975) and 300 – 400 Kcal according to BIS specifications (Anon, 1969). Composite mix in the present investigation provides 393 – 408 Kcal, thus satisfying the recommendations. Thus, 100 g of composite mix will be sufficient to meet 1/5th to 1/6th of the energy intake of the recommended dietary allowances.

Good quality protein provides all the essential amino acids in required proportion. The composition of the mixes or formulations is the results of the composition of ingredients used. Higher amount of calcium in skimmed milk powder, good amount of micronutrients in the Italian millet could have been a reason for the raised levels of these micronutrients in the mixes. Zinc is also an important element performing a range of functions in the body as it is a co-factor for a number of enzymes. The adverse effects of zinc deficiency are most prominent in tissues with rapid turnover, especially the immune system (Dibley, 2001). Since the Recommended Dietary Allowances (RDA) for zinc is 15 mg/day for an adult. Thus, addition of functional ingredients has increased the zinc content and it can be stated that 100 g of composite mix is sufficient to meet 1/4th of the Recommended Dietary Allowances.

Non-nutrient components

Tannins are condensed polyphenoilc compounds. A typical Indian diet based on cereals, legumes, vegetables and spices may contain 2-3 g of tannins (Gopalan*et al.*, 2004). Chetana (2008) reported tannin content as 119.27 mg in composite mix I (pearl millet, sorghum, green gram and soybean). In the present study, tannin content of the composite mix was 111.50 mg (Table 2).

Phytic acid is an important storage compounds in seeds including cereals and legumes. Phytates are thought to exhibit antioxidant properties by sequestering the iron before it can be involved in damaging processes, thus protecting against degenerative diseases. Pendelton (2004) have reported that the phytates have been shown to reduce the digestion of starch and reduce the overall glycemic index of the foods and also decrease cholesterol and triglycerides production by the liver. The formulated composite mix had about 3.10 mg of phytic acid. Higher levels of phytic acid content *i.e.* 231,186 and 86 (mg/100 g) in food mixture containing rice, defatted soy flour, SMP and tomato pulp (2:1:1:1) was reported by Rani and Khetarpaul (1999). They also found 269, 208 and 104 mg/100 g of phytic acid in raw, autoclaved and probiotic fermented food blends prepared with wheat, pigeon pea, SMP and tomato pulp (2:1:1:1) in 2003. The polyphenol content of the composite mix was 120 mg/100g. Similar trend in the polyphenol content of indigenous food mixture containing rice, defatted soy flour, skimmed milk powder and tomato pulp (2:1:1:1) was reported by Rani and Ketarpaul (1999, 2003).

IVPD

The *in vitro* protein digestibility in composite mix was found to be (55.3). This might due to the addition of defatted soy flour, roasted bengal gram dal and skimmed milk powder which might have helped to increase the *in vitro* protein digestibility. Anwara (2009) also documented the IVPD in different horse gram genotypes (38-68). This parameter is helpful to know the quality of protein being present in the food matrix.

pH and acidity

The pH and acidity of the composite mix on initial day and after 30 and 60 days of storage were analyzed (Table 3). pH and acidity (percentage) of the control CM, CMSP and CMMP declined over the storage period, whereas acidity (percentage) increased during storage period in all the three composite mixes. Glucose get converted to lactic acid in presence of probiotic organisms, which is might be responsible for the decline in pH in the present study.

Table 3: Changes in pH of composite mixes during storage

Food mixes	Initial	30 Days	60 Days
CM (control)	6.12	5.82	5.55
CMSP	5.25	5.10	4.98
CMMP	5.31	5.21	5.10
F-Value	NS	NS	NS
SEm±	32.27	32.23	32.20
CD at 5%	74.41	74.34	74.27

NS: Non Significant

Table 4: Changes in acidity (% lactic acid) of composite mixes during storage

Food mixes	Initial	30 Days	60 Days
CM (control)	1.59	1.65	1.74
CMSP	2.63	2.69	2.73
CMMP	2.19	2.28	2.41
F-Value	NS	NS	NS
SEm±	31.96	31.96	31.96
CD at 5%	73.70	73.70	73.70

NS: Non-Significant CM: Composite Mix, CMSP: Composite Mix with Single culture Probiotic (*Lactic acid bacillus*), CMMP: Composite Mix with Mixed culture Probiotic (*Streptococcus faecalis Clostridium butyricum, Bacillus mesentericu* sand *Lactic acid bacillus*).

A rapid drop in pH with corresponding increase in titrable acidity has been reported in cereals and legumes earlier (Agte *et al*., 1997) and *Lactobacillus acidophilus* fermented food mixture containing cereal, legume, skim milk powder and fresh tomato pulp (Rani and Khetarpaul, 1998). The acidity is correlated with the pH value and corresponding increase in acidity value in the CMSP and CMMP was due to formation of lactic acid and other acids by probiotic organisms (Table 4). Similar trend with regard to pH and acidity of raw and fermented mixes was observed by Sindhu and Khetarpaul (2000, 2004).

Conclusion

The results on evaluation of probiotic composite mixes indicate that CMSP *i.e*., composite mix with single probiotic culture is suitable for the development of products since it is rich in macro and micro nutrients and good IVPD. The mixesdeveloped were also found to be economical since the cost of preparation is less compared to the probiotic milk based products available in the market. Thus, development of food products from such mixes will not only provide nutrients but will also provide therapeutic benefits.

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