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FORMULATION OF SYNBIOTIC DRINK TO ENHANCE INTESTINAL GUT FLORA

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ABSTRACT

Keywords:

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The transition in diet has led to an increase in lifestyle disorders and thereby increased dependency on wonder drugs. With increased drug discovery and marketing, there is a compelling trend towards large drug dosage. However, food is judged not only for its nutritive properties but also for its ability to improve the health and well being of consumers, thus leading to the advent of "FUNCTIONAL FOODS". Probiotic organisms of the gut and prebiotics are two under explored functional foods. Hence the current study was done with the aim of formulating a synbiotic health drink to enhance the intestinal flora. Dietary prebiotic sources like whole wheat, oats, soyabean, samai and artichoke were used in the preparation of the health drink. Whole wheat, samai and soyabean was malted separately, shade dried and powdered. Standard procedure was followed while preparing artichoke powder. All the selected ingredients were blended together along with one gram of lyophilized vacuum dried lactic acid bacillus. Six variations of the drink was prepared and organoleptically evaluated to find the most accepted variation. On sensory evaluation, Variation II was graded to be very good in all aspects like appearance, taste, texture, colour and flavour. Ten grams of the best accepted variation II contained 4.7g of malted soyabean, 2.3g of oats powder, 1.2g each of malted wheat and malted samai and 0.5g of artichoke. The above variation also provided more energy (37.84 Kcal), protein (2.58g), fibre (1.23g) and less fat (0.07g). The nutrient content was analysed in the laboratory for energy, protein, fat, fibre and inulin (3.65g) content. The drink when supplemented was also found to reduce the faecal pathogenic load. Hence it can be concluded that the formulated synbiotic drink was low cost, nutritionally rich and also enhanced the intestinal flora. This drink can sure be considered as a potential nutritional supplement and a ideal alternative to drugs.

INTRODUCTION: Global urbanization has led to a nutrition transition leading to a clear shift from a diet rich in fibre, minerals and vitamins to one rich in carbohydrates and fats that has led to an increase in lifestyle disorders and thereby increased dependency

on wonder drugs. The global pharmaceutical industry is changing rapidly and the industry is being forced to look for new models of efficiency. India's pharmaceutical industry is now the third largest in the world in terms of volume. Its rank is 14th in terms of

value. Between September 2008 and September 2009, the total turnover of India's pharmaceuticals industry was US\$ 21.04 billion. The domestic market was worth US\$ 12.26 billion as reported by the Department of Pharmaceuticals, Ministry of Chemicals and Fertilizers. As per a report by IMS Health India, the Indian pharmaceutical market reached US\$ 10.04 billion in size in July 2010. A highly organized sector, the Indian Pharma Industry is estimated to be worth \$ 4.5 billion, growing at about 8 to 9 percent annually. The pharmaceutical industry in India meets around 70% of the country's demand for bulk drugs, drug intermediates, pharmaceutical formulations, chemicals, tablets, capsules, orals and injectable. With increased drug discovery and marketing, there is a compelling trend towards large drug dosage ¹.

To combat these trends, the World Health Organization advocated the implementation of alternative disease control strategies such as exploiting the therapeutic and prophylactic potential of probiotic bacteria and prebiotics. Food is no longer judged only in terms of taste and immediate nutritional needs, but in terms of their ability to improve the health and well being of consumers, thus leading to the advent of "FUNCTIONAL FOODS".

The term functional foods comprise some bacterial strains and products of plant and animal origin containing physiologically active compounds, beneficial for human health and reducing the risk of chronic diseases ². Among the best known functional compounds are probiotics and prebiotics.

Probiotics are traditionally defined as viable micro organisms that have a beneficial effect in the prevention and treatment of specific pathologic conditions when they are ingested in the stomach. Currently lactic acid bacteria, particularly *Lactobacillus* and *Bifidobacterium* species are well known probiotics. Probiotics enhance human health by stimulating immunity, competing for limited nutrients, inhibiting epithelial and mucosal adherence and inhibiting the production of antimicrobial substances ³.

Some probiotic strains have proven beneficial effects on the intestinal immunity. Sherwood (2003) proved that probiotics increase the number of Immunoglobulin A and other immunoglobulin

secreting cells in the intestinal mucosa thereby giving a boost to the immune system. It has been used successively in antibiotic associated diarrhoea, HIV disorder, enteral feeding diarrhoea, *Helicobacter pylori* infections, lactose intolerance, inflammatory bowel disease and some types of cancer ⁴.

Probiotic organisms are closely associated with the host's health as they act as an important biodefence in preventing colonization and proliferation of pathogenic bacteria in the intestine ⁵. Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon ⁶. Prebiotics enhances the fermentation and colonization of friendly bacteria like lactic acid bacteria and *Bifidobacterium bifidus*. It comes in various forms like inulin, fructooligosaccharide, galactooligo- saccharides of which inulin is the most common.

Saccharides which resist hydrolysis by digestive enzymes are not absorbed in the upper gastrointestinal tract. They pass into the large intestine where they modify the colonic micro biota so as to increase the load of potentially health promoting benefits ⁷. Foods containing oligosaccharides and polysaccharides are claimed to have prebiotic activity.

Among the identified prebiotic components, inulin is found as a natural component in several edible fruits and vegetables. The average daily consumption has been estimated to be one to four grams. Inulin is a group of fructose polymers (fructans) linked by β (2-1) bonds that limit their digestion by the upper intestinal enzymes. Inulin is found in plant species like whole wheat, raw oats, soyabean, onion, banana, chicory, artichoke and unrefined barley ⁸.

Inulin type fructan primarily enhance calcium absorption and had an effect on colonic absorption in humans ⁹. Probiotics, mainly active in the small intestine and prebiotics effective in the large intestine is said to produce a synergistic effect when administered in combination ¹⁰. The term synbiotic is used when a product contains both probiotics and prebiotic functional foods in it.

The non digestible fibres (prebiotics) are known to aid in faster fermentation and colonization of the friendly probiotic bacteria, thus bringing about a doubly

synergistic effect to the human host. The gut microflora is known to breakdown vitamins and also ferments fibre and carbohydrates to short chain fatty acids, which are important for supporting a healthy intestinal barrier and inhibit the growth of harmful bacteria. A healthy intestinal flora is also associated with intestinal (stool) regularity¹¹. The adult intestinal tract contains about four pounds of bacteria. Normally there should be a balance of about 85 percent probiotic bacteria and 15 percent harmful bacteria. But for many people it is vice versa¹².

Therefore, interactions between a person and micro organisms in ones body can be crucial to an individual's health and wellbeing. Hence colonic microbiota can be modulated with a feasible working concept for the development of functional food component.

In order to modulate the colonic microbiota, the study was conducted with the aim of formulating a low cost synbiotic health drink using dietary sources of prebiotics like malted soyabean, malted wheat, oats, malted samai and artichoke, which are an excellent source of fibre, palatable and easily digestible and lactic acid bacillus. A health drink is commonly consumed and preferred by all ages. It is easy to swallow and the burden of mastication is eliminated.

METHODOLOGY:

Selection of Prebiotic Foods: The ingredients for the formulation of the health drink shown in figure 1 was selectively chosen as they are all dietary sources of prebiotics and also rich in fibre. The following ingredients were used in the formulation of the prebiotic health drink

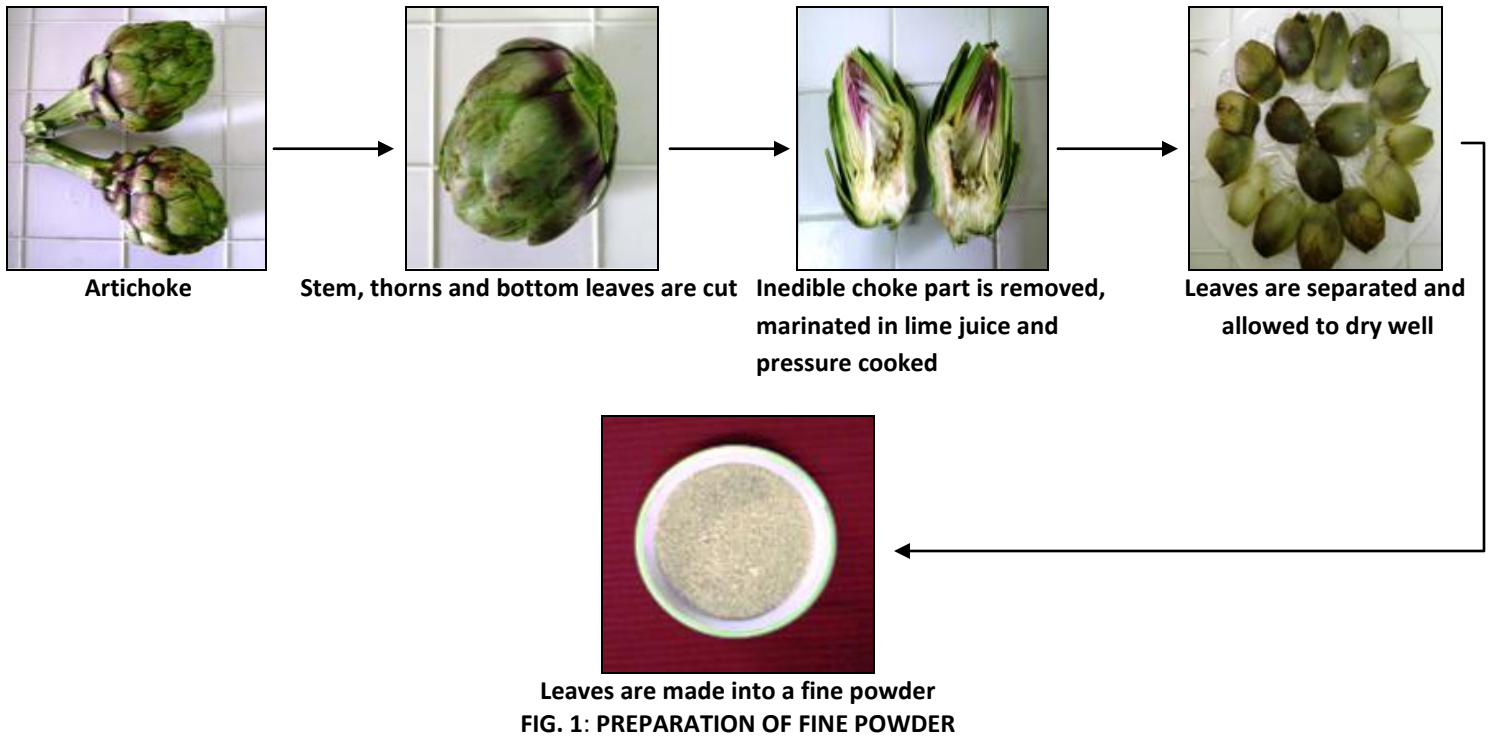
1. **Soya bean:** Soya bean is a natural gift rich in protein, an excellent source of fibre and is patented to have a prebiotic effect. Soymilk contains the prebiotic sugars stachyose and raffinose, which is easily digested by lactose intolerant people¹³. The hypocholesterolemic effect of soyabean has also been well documented by Zhan (2005)¹⁴. Because of these nutritional properties, malted soyabean forms a major ingredient in the formulated prebiotic health mix. Soya beans were sprouted for 72 hours and malted. It was then incorporated in different proportions to make six variations.

2. **Oats:** Oats is one of the few foods that contain high levels of soluble fibre, which comprise of a polysaccharide known as β - D glucan¹⁵. This prebiotic ingredient is associated with better gut health, increasing the absorption of calcium and magnesium and lowering blood glucose and cholesterol levels¹⁶. A study conducted by Stephen *et al.*, (1997)¹⁷ concluded that oat fibre provides for stool bulking and thus reduces constipation. Keeping this in mind, varied proportion of oat flakes powder was used in the different variations.

3. **Whole wheat:** Whole wheat was chosen, as it is a staple cereal in India and is an excellent source of fibre. Whole grain cereals are known to reduce the risk of coronary heart disease and the fibre in it helps in preventing constipation. Whole wheat was sprouted for 30 hours, shade dried and then powdered. The prebiotic property of whole wheat is well documented by Costabile *et al.*, (2007)¹⁸, that daily consumption of 48g whole grain wheat as breakfast exerted a pronounced prebiotic effect on the human gut microbiota composition. Hence, the investigator selected this ingredient for the health drink.

4. **Samai:** Samai is minor millet consumed in the tribal community in India. It is a good source of energy, protein, iron and dietary fibre due to which it has conferred health benefits¹⁹. Samai was sprouted for 24 hours, shade dried and then powdered in order to dissolve properly in the milk without leaving any residue.

5. **Artichoke:** Artichoke has been traditionally used as food and medicinal herb for its diuretic and digestive properties. It has been widely used in connection with gastrointestinal complaints¹⁹. It is an excellent source of dietary fibre providing about 10g/100g. According to food and drug administration, artichoke provides about 20 percent or more of the daily value of fibre²⁰. In the first step, stem, thorns and discoloured leaves at the bottom were cut. In the second step, it was marinated in lime juice and pressure cooked for 10 minutes and dried well. The leaves were then put into a blender to make a fine powder as shown in **figure 1**.



Formulation of Prebiotic Health Drink: Soya bean, whole wheat and samai were washed in running water and soaked in three times its volume of water for 24 hours. They were then kept in wet muslin cloth for sprouting to about 1centimeter. The sprouting time for soya bean, whole wheat and samai were 72 hours, 30 hours and 24 hours respectively. They were then shade dried in order to avoid nutrient loss and powdered. Oats and artichoke were also powdered separately.

Malting process was done in order to improve its digestible properties, make it more palatable and easy to consume. The process of sprouting also enhances the nutritive value of the ingredients, improves its bioavailability and destroys the anti nutritional factors present in food. Therefore soyabean, whole wheat and samai were sprouted and malted.

Development of Different Variations: To test the acceptability of the health drink, six variations were prepared. The ingredients were mixed in various proportions to develop six variations. However, the quantity of artichoke was kept constant at 0.5 g for all the developed variations.

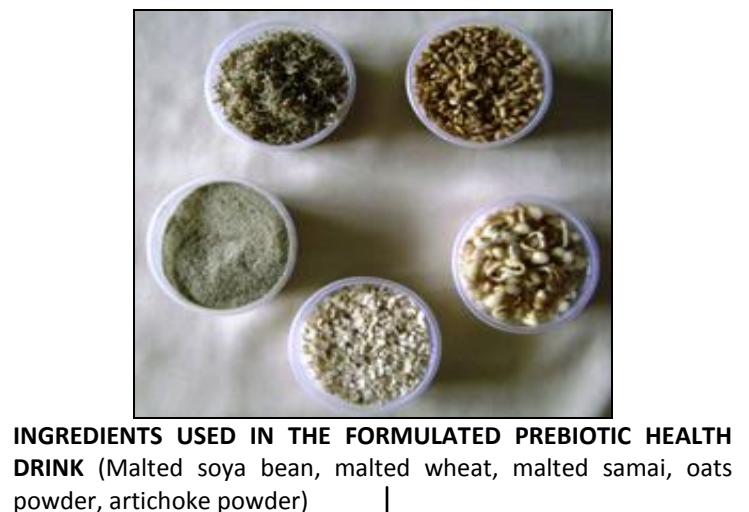


FIG. 2: FORMULATION OF THE PREBIOTIC HEALTH DRINK

Organoleptic Evaluation: Organoleptic evaluation was carried out by a panel of 30 members to test the acceptability in terms of color, flavor, texture, taste and appearance. The panel included 12 males and 18 females in the age group of 40 - 65 years. A hedonic rating scale relates to pleasurable or unpleasurable experiences. Each attribute was rated on a scale of excellent, very good, good, fair and poor and key scores 5, 4, 3, 2 and 1 were given respectively and the mean scores were calculated. A five point rating scale is usually used to measure the consumer acceptability of the food products²¹.

Nutrient analysis of the Formulated Health Drink: The formulated health drink was chemically analysed for nutrients like proteins, fats, carbohydrates, fibre and inulin. Energy was calculated by using the Atwater method. The inulin content was also analysed, as it is a prebiotic component.

Synbiotic Drink: The synbiotic drink was prepared using ingredients like malted soyabean, oats flakes powder, malted wheat, malted samai and artichoke powder in the above mentioned proportion along with one gram of lactic acid bacillus species containing 150 millions spores.

RESULTS AND DISCUSSIONS:

Development of Different Variations: Totally six variations were developed to standardize prebiotic drink using different ingredients at different proportions as shown in **Table 1**. Table 1 shows the quantity of the different ingredients used in the formulation of the six variations. The cost per serving for all the variations of the formulated health mix ranged from 52 paise- 76 paise.

Organoleptic evaluation: Organoleptic evaluation carried out to find the most acceptable variation is shown in **Table 2**.

TABLE 1: COMPOSITION OF DIFFERENT VARIATIONS OF FORMULATED HEALTH MIX

Variations	Soya flour (g)	Oats Powder (g)	Wheat Flour (g)	Samai Flour (g)	Artichoke Powder (g)	Cost (Paise)
I	2.3	2.3	1.2	1.2	0.5	54
II	4.7	2.3	1.2	1.2	0.5	62
III	1.2	2.3	4.7	1.2	0.5	57
IV	2.3	4.7	1.2	1.2	0.5	76
V	3.4	1.2	2.3	2.3	0.5	52
VI	1.2	3.4	1.2	3.4	0.5	67

TABLE 2: MEAN SCORES OF THE DEVELOPED VARIATIONS

Variations	Appearance	Colour	Flavour	Taste	Texture
I	3.1±0.56	3.35±0.5	3.3±0.5	3.25±0.71	3.25±0.51
II	3.6±0.5	3.35±0.5	3.75±0.51	3.85±0.52	3.55±0.5
III	3.2±0.51	3.45±0.5	3.75±0.51	3.45±0.52	3.35±0.5
IV	3.35±0.72	3.5±0.5	3.25±0.71	3.45±0.74	3.35±0.5
V	3.6±0.76	3.1±0.7	3.5±0.75	3.45±0.74	3.25±0.71
VI	2.8±0.71	2.7±0.72	2.75±0.71	2.45±0.75	2.7±0.72

The above table represents the mean scores for each attribute for all the six tested variations. In terms of colour, flavour, taste and texture, Variation II scored the maximum and Variation VI had the least score. The comments from the panel members showed that, Variation II tasted very good and had pleasant odour and flavour.

The appearance and texture was also scored excellent by majority of the panel members. Therefore, the second variation was considered the most acceptable. Ten grams of the best accepted variation II contained 4.7g of malted soyabean, 2.3g of oats powder, 1.2g each of malted wheat and malted samai and 0.5g of artichoke powder.

Therefore the second variation was considered the most acceptable and hence formulated for the supplementation study.

Computing the Nutritive Value for the different variations:

The nutritive value for all the six variations was calculated using the standard ICMR values²². Energy, protein, carbohydrates, fat and fibre was computed for all the developed variations. **Table 3** shows the computed nutritive value for all the six developed variations. When compared to all other variations, the second variation provides more energy, protein, fibre and less fat. Hence, it was considered nutrient rich and so was further chemically analysed for the nutrients present in it.

Nutrient analysis of the Formulated Health Drink: nutrients like protein, fat, carbohydrates, fibre and Table 4 shows the chemically analysed values for inulin.

TABLE 3: COMPUTED NUTRITIVE VALUE OF THE DEVELOPED VARIATIONS

VARIATIONS	ENERGY (Kcals) (g)	PROTEINS (g)	FATS (g)	CARBOHYDRATES (g)	FIBRE (g)
I	36.65	1.87	0.74	5.3	1.18
II	37.84	2.58	0.07	4.19	1.23
III	34.65	1.49	0.53	5.89	1.16
IV	37.18	1.91	0.88	5.16	0.41
V	36.35	2.15	0.92	4.76	0.44
VI	34.68	1.35	0.70	5.65	1.98

TABLE 4: ANALYZED VALUE FOR THE SELECTED VARIATION II

NUTRIENTS	METHOD	NUTRITIVE VALUE (100 g)
Energy (kcal)	At water	57.4
Proteins (g)	Nitrogen-micro kjeldhal	14.35
Carbohydrates (g)	Phenol sulphuric acid	46.0
Fats (g)	Soxhlet	33.3
Inulin (g)	Test for inulin	3.65
Fibre (g)	Nin method	3.26

The energy, proteins, carbohydrates, fats, fibre and inulin value for 100 grams of the health drink are clearly shown in Table 4. 100 grams of the health mix provides 57 Kcals of energy and contains 14.35 g and 33.3g of protein and fat respectively.

The inulin content of health mix is 3.65g. Inulin is a non digestible food ingredient that selectively stimulates growth of a number of potentially health stimulating bacteria and has well documented evidence in the prevention and treatment of intestinal diseases and hence improve the colonic function²³.

Energy contribution for the best accepted variation II by carbohydrates, fats, proteins were calculated by proximate principle as shown in table 4.

Faecal Pathogenic Load: The effect of the formulated synbiotic drink on faecal pathogenic load was tested and showed that two samples had *salmonella* and *shigella* in moderate amount in the faeces. However, after one month of supplementation with the synbiotic preparation, these pathogens were completely eliminated from their gastrointestinal tract as shown in the below figure 3.

CONCLUSION: The field of functional foods especially probiotics and prebiotics is very promising. The formulated health drink is nutritious, low cost and can confer a shoal of health benefits to individuals. It also can open up a large market potential in terms of functional food products in the future.

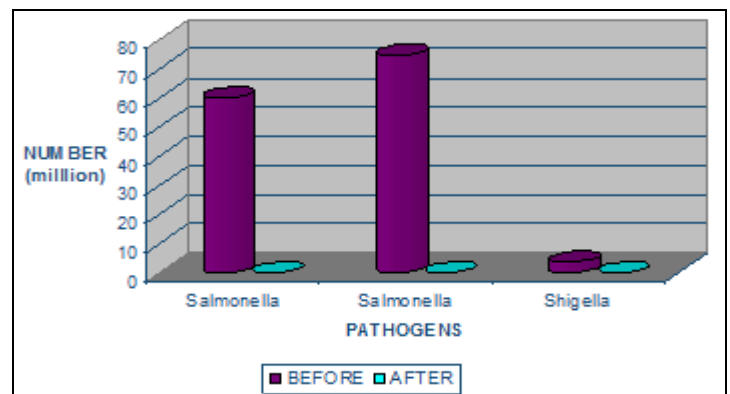


FIG. 3: FAECAL PATHOGENIC LOAD

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