Assessment of pulse extracts as Potential Non-Dairy substrate for probiotication

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CERTIFICATE

This is to certify that the work entitled, "**Assessment of pulse extracts as a Potential Non-Dairy substrate for probiotication**" submitted by Kanishka Sinhal (071551) , Rushil Bansal (071559), Varun Dhir (071708) in fulfillment for the award of degree of Bachelor's of Technology in Biotechnology of Jaypee University of Information Technology has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

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LIST OF ABBREVIATIONS

1. CFU = Colony Forming Unit.
2. OD = Optical Density.
3. DDW = Double Distilled Water.
4. DH_2O = Distilled Water.
5. Std. = Standard.
6. Conc. = Concentration.
7. Min = Minutes.
8. Temp = Temperature.
9. Hrs = Hours.
10. Sp. = Species

ABSTRACT

The Aim of Project was assessment of pulse extracts as a Potential Non-Dairy substrate for probiotication. One of the most promising areas of development in the area of functional foods has been the use of probiotics and their role in human health and disease. There are a number of possible means by which probiotics may alter health; one of those putative effects is the alteration of immune function. Probiotic bacteria are sold mainly in fermented and dairy products play a predominant role as carriers of probiotics.

These foods are well suited to promoting the positive health image of probiotics for several reasons: *1*) Dairy products in particular, already have a positive health image; *2*) consumers are familiar with the fact that fermented foods and dairy product contain living microorganisms (bacteria); and *3*) probiotics used as starter organisms combine the positive images of fermentation and probiotic cultures. When probiotics are added to fermented foods, several factors must be considered that may influence the ability of the probiotics to survive in the product and become active when entering the consumer's gastrointestinal tract

Much of the research work in probiotics has focused on the development of probiotic dairy products such as yogurt or freeze-dried cultures. From past many years dairy probiotic product are being used all around the globe although the allergy to dairy products affect negatively some person. Lactose intolerance and the cholesterol content are two major drawbacks related to the fermented dairy products. Traditions and economic reasons that limit the use of dairy fermented product in some developing countries promote the idea of reduction of milk components as vehicles for the probiotic agents. There are very few reports of human intervention studies with Non-Dairy probiotics. Therefore the aim of the study is to develop the Non-Dairy substrate for prebiotication.

In this process we extracted the nutrients from the pulses, estimated the total carbohydrate content, Estimated the protein content of the pulse extracts, Evaluated the suitability of the selected extracts for growth of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* by calculating the CFU/ml.

CHAPTER 1

INTRODUCTION

Aim is assessment of pulse extracts as a Potential Non-Dairy substrate for probiotication

1.1 Functional food

- **Functional food** or **medicinal food** is any fresh or processed food claimed to have a health-promoting or disease-preventing property beyond the basic function of supplying nutrients. The general category of functional foods includes processed food or foods fortified with health-promoting additives, like "vitamin-enriched" products, Probiotic food (Agriculture and Agri-Food Canada).
- A functional ingredient can be defined as 'a dietary ingredient that affects its host in a targeted manner so as to exert positive effects that may, in due course, justify certain health claims.
- In other words, foods containing these ingredients (functional foods) are foods that have health promoting properties over and above their nutritional value.
- The term was first used in Japan in the 1980s where there is a government approval process for functional foods called Foods for Specified Health Use (FOSHU)

Functional Food is of three types

a) Prebiotic

b) Probiotic

c) Synbiotic

1.2 Prebiotic

- A prebiotic is 'a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, which can improve host health' (British Nutrition Foundation, 2004).
- They occur naturally in some foods such as chicory, artichoke, leeks, onions and asparagus Oat meal
- Prebiotics help feed the good bacteria already in the digestive system.
- They can improve the health of the digestive system and they may also help to strengthen the immune system.

Example: - Inulin and Fructooligosaccharides (FOS).

1.3 Synbiotic

- Appropriate combinations of pre- and probiotics are synbiotics. As probiotics are mainly active in the small intestine and prebiotics are only effective in the large intestine (Roberfroid et al., 1995) [the] combination of the two may give a synergistic effect.
- Examples :-

1. Fermented milks (yogurt and kefir)
2. *Bifidobacteria* and Fructo-oligosaccharides (FOS)
3. *Lactobacillus* GG and Inulins
4. *Bifidobacteria* and *Lactobacilli* with FOS or Inulins.

1.4 Probiotic

The term "probiotics" was first introduced in 1953 by Kollath. Earlier probiotics were defined as microbial derived factors that stimulate the growth of other microorganisms. Probiotics are dietary supplements of live bacteria or yeasts thought to be healthy for the host organism. According to the currently adopted definition by FAO/WHO, probiotics are: *'Live microorganisms which when administered in adequate amounts confer a health benefit on the host'* (FAO/WHO, 2001)

Important Probiotic Organisms:

Bacteria: - *Lactobacillus sp, Bifidobacterium sp and Lactococcus sp.*

Yeast: - *Saccharomyces boulardii.*

1.5 History of Probiotic Food

1. The concept of probiotics evolved around **1900**, when Nobel Prize-winning Elie Metchnikoff hypothesized that the long, healthy lives of Bulgarian peasants were the result of their consumption of fermented milk products and later he was convinced that yogurt contained the organisms necessary to protect the intestine from the damaging effects of other harmful bacteria.

2. The first clinical trials were performed in the **1930s** on the effect of probiotics on constipation.

3. In the **1950s**, a probiotic product was licensed by the United States Department of Agriculture as a drug for the treatment of scour (Escherichia coli infection) among pigs

Over the last century, different micro-organisms have been used for their ability to prevent and cure diseases, leading to the coining of the term probiotics

4. The discovery by Mann and Spoerig in the **1974** that people who drank yogurt fermented with wild strains of Lactobacillus sp. had very low values for blood serum cholesterol opened up a new area of study.

5. Harrison et al. **1975** reported that cells of Lactobacillus acidophilus added to infant formula decreased levels of serum cholesterol.

6. Gilliland et al. in the **1985**, Buck and Gilliland in the **1994**, and Gilliland and Walker in the **1989**, Gill and Guarner in the **2004**, showed control of serum cholesterol levels in adult human experiments.

7. In **1994**, the World Health Organization deemed probiotics to be the next-most important immune defense system when commonly prescribed antibiotics are rendered useless by antibiotic resistance. The use of probiotics in antibiotic resistance is termed as a microbial interference therapy.

1.6 Advantages of Probiotic Food

Alleviation of lactose intolerance Lactose intolerance is a problem for 70% of the world's population who have a low amount of intestinal b-galactosidase activity and for whom lactose behaves like an osmotic, nondigestible carbohydrate. Probiotics have been shown to improve lactose digestion by reducing the intolerance symptoms as well as by slowing orocecal transit (Roberfroid B, 2000).

Immune enhancement Infants supplemented with a strain of *Lactobacillus casei* have enhanced concentrations of circulating immunoglobulin A (IgA), which correlates with shortened duration of rotavirus-induced diarrhea. In addition, consumption of *L. acidophilus* and

Bifidobacterium bifidum significantly enhances the nonspecific immune phagocytic activity of circulating blood granulocytes. This latter effect may explain, in part, the stimulation of intestinal IgA antibody responses in infants. Indeed, it is known that phagocytic activity is involved with natural immunity and that phagocytes are implicated in antibody immune responses acting as antigen-presenting cells. Finally, ingestion of yogurt has been shown to stimulate the production of cytokines by blood mononuclear cells (Roberfroid B, 2000).

Decrease in fecal enzymes and mutagenicity Probiotic lactobacilli and bifidobacteria strains decrease the_quantity of such fecal microbial enzymes as b-glucuronidase,_b-glucosidase, nitroreductase, and urease, which are involved in the metabolic activation of miscellaneous mutagens and carcinogens. In addition, these or similar strains have been reported to decrease fecal and urinary mutagenicity in healthy volunteers consuming fried ground beef (Roberfroid B, 2000).

List of Probiotic Products (Senok, 2009)

Product no.	Product type	Types of probiotic species present	Organisms and No. (per suggested serving)	Claim of beneficial effect
1.	Fermented milk	1	*L. casei shirota* $10^{\times 10}$ or 6.5 billion per bottle	Healthy gut immunity
2	Yogurt	1	Bifidus essensis	Regulates the digestive process
3	Laban	1	Gefilac	Maintains favorable balance of bacteria in digestive system; fights harmful bacteria
4	Laban	2	*L. acidophilus* LA5; *B. bifidus* BB12	Improves digestion, boosts immunity

Table - 1.1

1.7 Non-Dairy Probiotics

1. ***Sauerkraut*** **:** a much loved and much loathed fermented cabbage dish hailing from northern Europe, naturally prepared sauerkraut is both tart and salty. Furthermore, the process of fermenting cabbage actually creates isothiocyanate – a substance thought to inhibit the formation of cancer and tumors.

2. ***Kombucha*** : a fermented tea thought to originate in Russia or China, kombucha has long been considered a health tonic. Kombucha, like other fermented foods and beverages, has a sour flavor with a taste reminiscent of apple cider vinegar combined with club soda, though home-brewed kombucha is often less acidic than storebought. A starter culture sometimes called a kombucha mushroom, mother or scoby (symbiotic culture of bacteria and yeasts) is necessary to prepare kombucha. Kombucha, like other fermented foods and beverages, is rich in beneficial bacteria and vitamin B12. It also contains a substance called glucaric acid. Glucaric acid is deeply detoxifying and recent research indicates great promise that glucaric acid is effective in the treatment and prevention of cancer.

3. ***Miso*** : composed of soybeans in combination with barley or rice. Miso is primarily fermented by aspergillus oryzae, a mold that is also responsible for the transformation of soybeans into shoyu or tamari. Miso is high in vitamin K (learn about vitamin K and other (fat soluble vitamins) as well as vitamin B6. It's also a good source of phosphorus, manganese and zinc. Zinc, in particular, is essential for proper immune system function.

4. ***Water Kefir*** : water kefir, alternatively known as tibicos and Japanese water crystals, is a probiotic beverage. Water kefir grains are translucent and gelatinous with a crystal-like appearance. Water kefir grains are a symbiotic culture of bacteria and yeasts including lactobacillus hilgardii.

5. ***Moroccan Preserved Lemons*** : moroccan preserved lemons are naturally fermented without the use of a starter – just benign bacteria and yeasts naturally present in the air, on our skin and on the fruits themselves. Lemons, like all citrus, are rich in antioxidants and vitamin C in particular.

6. ***Coconut Kefir*** : coconut kefir is a probiotic beverage prepared from young coconut water and a starter culture. Coconut kefir combines many of the benefits of coconut with the

benefits of probiotics. Coconut water is rich in minerals like calcium and potassium, but it is relatively sweet. By introducing beneficial bacteria into the fresh coconut water, the bacteria metabolize its sugars and produce lactic and acetic acids which lower the overall glycemic index of the beverage.

7. ***Ginger Beer*** **:** traditional ginger beer is cultured using a symbiotic culture of bacteria and yeasts. Powdered ginger and sugar mixed together to encourage the growth of wild bacteria and yeasts and this ginger bug is introduced into sugar water to and allowed to continue to brew.

1.8 Pulses

Dried legumes and their edible seeds, known as pulses, are classified into three groups - lentils, beans and peas. Legumes and Pulses are a natural source of protein, high in fibre and low in fat. They have a wide range of flavours and textures and form a large part of the Indian daily diet.

They are eaten either whole (with the skin still intact) or split in half (with or without their skins). The term "dal"or "daal" refers to a bean or lentil which has had the outer husk removed and the remaining lentil is then split. This ensures a quicker cooking time and a softer, creamier texture when cooked.

1. The **Chick peas**(***Cicer arietinum***) (also **garbanzo bean, chana** (north India), **Indian pea, ceci bean, Bengal gram**) is an edible legume of the family Fabaceae, subfamily Faboideae. Chickpeas are high in protein and one of the earliest cultivated vegetables; 7,500-year-old remains have been found in the Middle East.

Figure 1

Health Benefits of Chick peas

Garbanzo beans (chickpeas) provide an excellent source of molybdenum. They are a very good source of folic acid, fiber, and manganese. They are also a good source of protein, as well as minerals such as iron, copper, zinc, and magnesium. As a good source of fiber, garbanzo beans can help lower cholesterol and improve blood sugar levels. This makes them a great food especially for diabetics and insulin-resistant individuals. When served with high quality grains, garbanzo beans are an extremely-low-fat, complete protein food.

Garbanzo beans contain molybdenum which is a trace mineral that is needed for the body's mechanism to detoxify sulfite's. sulfites are a preservative commonly found in wine, luncheon meats, and fresh salad in most salad bars. Sulfite-sensitive Individuals who are deficient in molybdenum may experience headaches, a racing heartbeat, or confusion.

Caution: Garbanzo beans can cause a severe allergic reaction in those who are sensitive to them. They contain purines which can lead to excess accumulation of uric acid. Excess accumulation of uric acid may contribute to health problems, such as gout and kidney stones. Garbanzo beans also contain large amounts of oxalate. Individuals who have a history of oxalate-containing kidney stones should avoid over consuming them.

2. The **Rajma(Red kidney Beans, *Phaseolus vulgaris*)** with its dark red skin is named for its visual resemblance to a kidney. The kidney bean is also known as the red bean, although this usage can cause confusion with other red beans. Red kidney beans (Rājmā in Hindi and Punjabi) are an integral part of the cuisine in northern region of India.

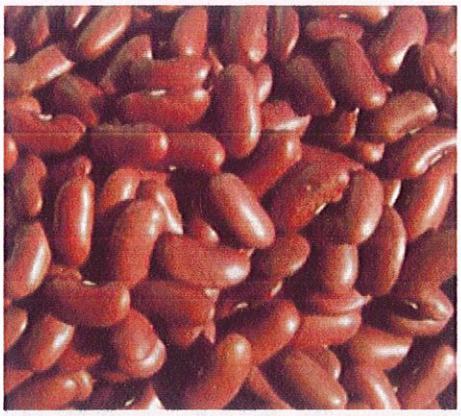


Figure 2

Health Benefits of Kidney Beans-Rajma

Kidney bean (aka common beans) is a highly nutritious variety of herbaceous plant that is very popular in countries like India, China, Brazil and America. In fact, the plant is considered as one of the major foundations of Native American agriculture and forms an important part of the cultural cuisine of the country. Kidney beans are classified under the botanical name, Phaseolus vulgaris and belong to the plant family Fabaceae. Originating from a common bean ancestor in Peru, the plant species was introduced first in the southern and central zones of America, by migrating Indian traders and later in Europe, by certain Spanish explorers, during the 15th century. Subsequently, Africa and Asia became acquainted with kidney beans, as a result of migrating traders from Spain and Portugal. Today, these edible beans are available in both dried and canned form and are associated

with the prevention of a number of ailments and diseases. They are particularly known for their beneficial effects on the cardio-vascular system and digestive system.

3. **Lobiya/Chawli/Black Eye Peas*(Vigna unguiculata)*** These are a subspecies of the cowpea, grown for its medium-sized edible bean, which mutates easily giving rise to a number of varieties, the common commercial one called the California Blackeye being pale-colored with a prominent black spot. Quite popular in the west especially South America...it is often used in Indian Cooking as well.

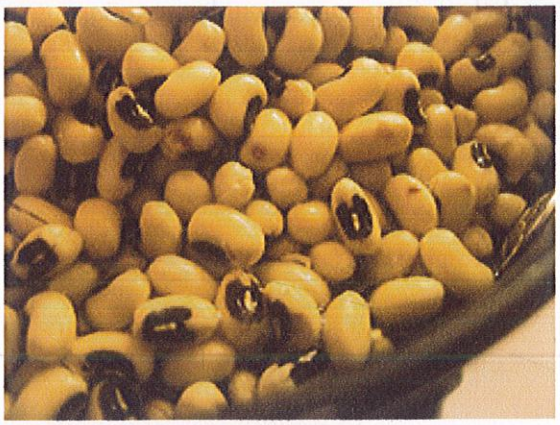


Figure 3

Health Benefits of Black Eye Peas

One of the main health benefits of black-eyed peas is their high fiber content. A 1/2 cup of dry black-eyed peas that are cooked have 5.6 g of fiber, while a 1/2 cup of canned black-eyed peas contains 4 g. These amounts will vary among brands of black-eyed peas. Fiber is a nutrient that helps regulate your digestive system, and increasing your intake could help alleviate constipation and symptoms of irritable bowel syndrome. Fiber also helps keep your cholesterol levels healthy by preventing cholesterol from being absorbed into your bloodstream, which reduces your risk of developing heart disease. Additionally, high-fiber

foods keep you feeling full, since they are digested slowly -- which is important for weight control.

4. **Masoor Dal(*Lens culinaris*)** While whole, this bean is greenish-brown, even though they can be prepared whole (masoor beans) Indian recipes often call for the skinned and split masoor, which is called masoor dal. Skinned split Masoor beans are actually called red lentils (orange in color).
They have a dark, earthy flavor and a creamy texture. These lentils pair well with tomatoes and kheema/mince meats, sausages, and may be served on their own as a side dish, or incorporated into soups, stews, salads and Indian dal.

Figure 4

Health Benefits of Masoor Dal

This lentil is good as a remedy when a person is facing dysentery problems. Masoor daal is good for the excretory system and helps keeping it clean. It is good for people facing illnesses due to impure blood.

Masoor daal reduces the growth of cough in the lungs and helps reduce acidity as well. Soup made of this lentil is given to a patient suffering from fever. It will give strength to the body of the patient as well as keep the blood pure. People who have piles and bleed as lot during excretion should definitely have masoor daal. It will help reduce this problem.

Powder made from masoor daal should be used instead of soap for infants and children. This helps restore the moisture in the skin of children. It brings a glow to skin and prevents it from cracking in the winter season. It is used as a face pack when people have very oily skin. It reduces the oil produced from the skin and helps reduce acne and pimples. It also prevents itching of skin.

5. **Mung dal/Moong Dal(whole and split, *Vigna Radiata*).** Whole moong is actually a bean or pulse and is known as 'sabat moong' . They are small green beans fairly used in India, China, Thailand and Japan. Sprouted they are used in salads or stir fries with lemon juice or vinaigrette.

In India Moong dal is used, which is split moong beans with the skin left (green skin yellow lentil) on or without the skin(yellow lentil). It is used to make delicious dals and curries.Moong lentils in particular is very easy to digest and take on seasonings and spices very well.

Health Benefits Of Mung Bean

1. Aids in weight loss

Moong dal or green beans provide great source of complex carbohydrates, fiber and protein. Also, they are an excellent source of molybdenum and folic acid. They provide a good nutrition for diet ers since they are low in fat.

2. Lowers cholesterol levels

Regular consumption of green beans helps to reduce cholesterol since they are rich in fiber. According to recent studies, high fiber in green beans keeps blood sugar from rising after mealtime. This makes green beans a great choice for people with insulin resistance, or hypoglycemia.

3. Promote heart health

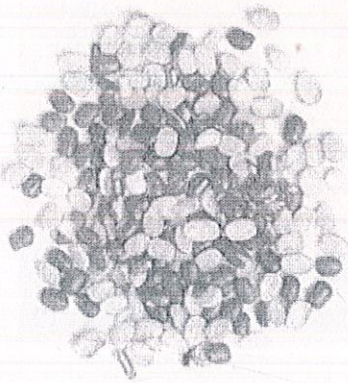
Green beans have antioxidants properties; folic acid, fiber, magnesium and vitamin B6 which help promote heart health. Vitamin B6 and folic acid lower homocysteine levels in the body, which is essential in a metabolic process known as methylation cycle. A high level of homocysteine in the blood is attributed to heart attack, peripheral vascular disease and stroke.

4. Prevent age related muscular diseases

They provide an excellent source of folates, a great nutrition during preconception. It prevents neural-tube defects during pregnancy.

Since moong dal or green bean is a low carb diet, studies show that it may have negative effects on health, especially with regards to weight loss.

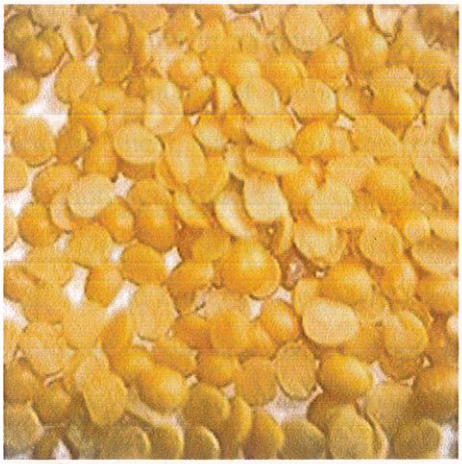
6. **Urad Dal (whole and Split, *Vigna Mungo*)** also called Black Beluga Lentils. Whole Urad dal/dahl is used more like a chili or stew than a soup or dal/dahl. These lentil-like beans have black skins covering creamy white interiors. Whole urad dal/dahl derive their strong, rich, earthy flavor from the black skins and have an uncanny ability to absorb flavors. Split and without the skin Urad dal is a white lentil used along with rice to make dosas, the crisp pancakes of southern India and other Rice preparations. In South India, Urad dal is used as a seasoning with mustard seeds for curries.



<u>Health benefits of Urad Dal</u>

It is beneficial and valuable in the treatment of following diseases Diabetes
Sexual dysfunction like impotency, premature ejaculation, thinness of semen
Nervous disorders like nervous weakness, weakness of memory, schizophrenia, hysteria
Digestive system disorders like dyspepsia, gastric, catarrah, dysentery, diarrhoea
Rheumatic afflictions like contracted knee, stiff shoulder

7. **Tur dal/Toor Dal/Tuvardal/Toovhar dal(*Cajanus cajan*)** is a glassy dark yellow split pea (pigeon pea), similar to chana dal. Toovar da exhibits a thick gelatinous/meaty consistency. They take a little longer to cook than moong or masoor dal/dahl. These yellow split peas can be made into dal which is served with side dish of vegetables, rice or flat breads. The South Indian delicacy, sambhar which is an accompaniment for dosa, idli or even rice is cooked with toordal.



Health Benefits of Tur Dal

It is very nutritious and is recommended for diabetics, as are other pulses. Though very beneficial in limited quantities excessive intake causes flatulence, which some sources claim can be prevented by adding a little asafoetida, pepper and ginger in the cultinary preparations. It is very popular in Punjabi cuisine of India and Pakistan where it is known as "maanh".

1.9 Literature survey

Probiotic :- a probiotic is a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract. Probiotics are widely used to prepare fermented dairy products such as yogurt or freeze-dried cultures. In the future, they may also be found in fermented vegetables and meats. Several health-related effects associated with the intake of probiotics, including alleviation of lactose intolerance and immune enhancement, have been reported in human studies. Some evidence suggests a role for probiotics in reducing the risk of rotavirus-induced diarrhea and colon cancer.

Prebiotic :- a prebiotic is defined as "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon. Modification by prebiotics of the composition of the colonic microflora leads to the predominance of a few of the potentially health-promoting bacteria, especially, but not exclusively, lactobacilli and bifidobacteria (Roberfroid B, 2000).

1.9.1 Probiotic formulation

Probiotic formulation should satisfy two requisites :-

1. It should be able to deliver adequate number of viable microorganisms to the target organ.
2. It should have a sufficiently long shelf life.

A number of probiotic products are marketed in different dosage forms such as powders, tablets, capsules and food products such as yoghurt and ice creams. They are available in combination with various vitamins and antibiotics.

Bacillus coagulans (B. coagulans), commonly mislabeled as *Lactobacillus sporogenes,* has a long history of use as a probiotic. Apart from dietary supplement, bacillus probiotics are

used as a therapeutic product for the treatment of gastrointestinal and urinary tract infections. The

therapeutic benefit is partly due to the ability of *B. coagulans* to secrete a bacteriocin, coagulin, which is active against a broad spectrum of enteric microbes (Bansal K, et.al 2009).

Most probiotics fall into the group of organisms' known as lactic acid-producing bacteria and are normally consumed in the form of yogurt, fermented milks or other fermented foods. Some of the beneficial effects of lactic acid bacteria consumption include:

(i) Improving intestinal tract health

(ii) Enhancing the immune system, synthesizing and enhancing the bioavailability of nutrients

(iii) Reducing symptoms of lactose intolerance, decreasing the prevalence of allergy in susceptible individuals.

(iv) Reducing risk of certain cancers.

The mechanisms by which probiotics exert their effects are largely unknown, but may involve modifying gut pH, antagonizing pathogens through production of antimicrobial compounds, competing for pathogen binding and receptor sites as well as for available nutrients and growth factors, stimulating immunomodulatory cells, and producing lactase. (Parvez S, et.al 2006)

The role(s) of probiotics bacteria in dairy fermentations is to assist in (Parvez S, et.al 2006)

(i) The preservation of the milk by the generation of lactic acid and possibly antimicrobial compounds

(ii) The production of flavour compounds (e.g. acetaldehyde in yoghurt and cheese) and other metabolites (e.g. extracellular polysaccharides) that will provide a product with the organoleptic properties desired by the consumer

(iii) To improve the nutritional value of food, as in, for example, the release of free amino acids or the synthesis of vitamins

(iv) The provision of special therapeutic or prophylactic properties as cancer

Various health benefits from probiotics consumption (Parvez S, et.al 2006)

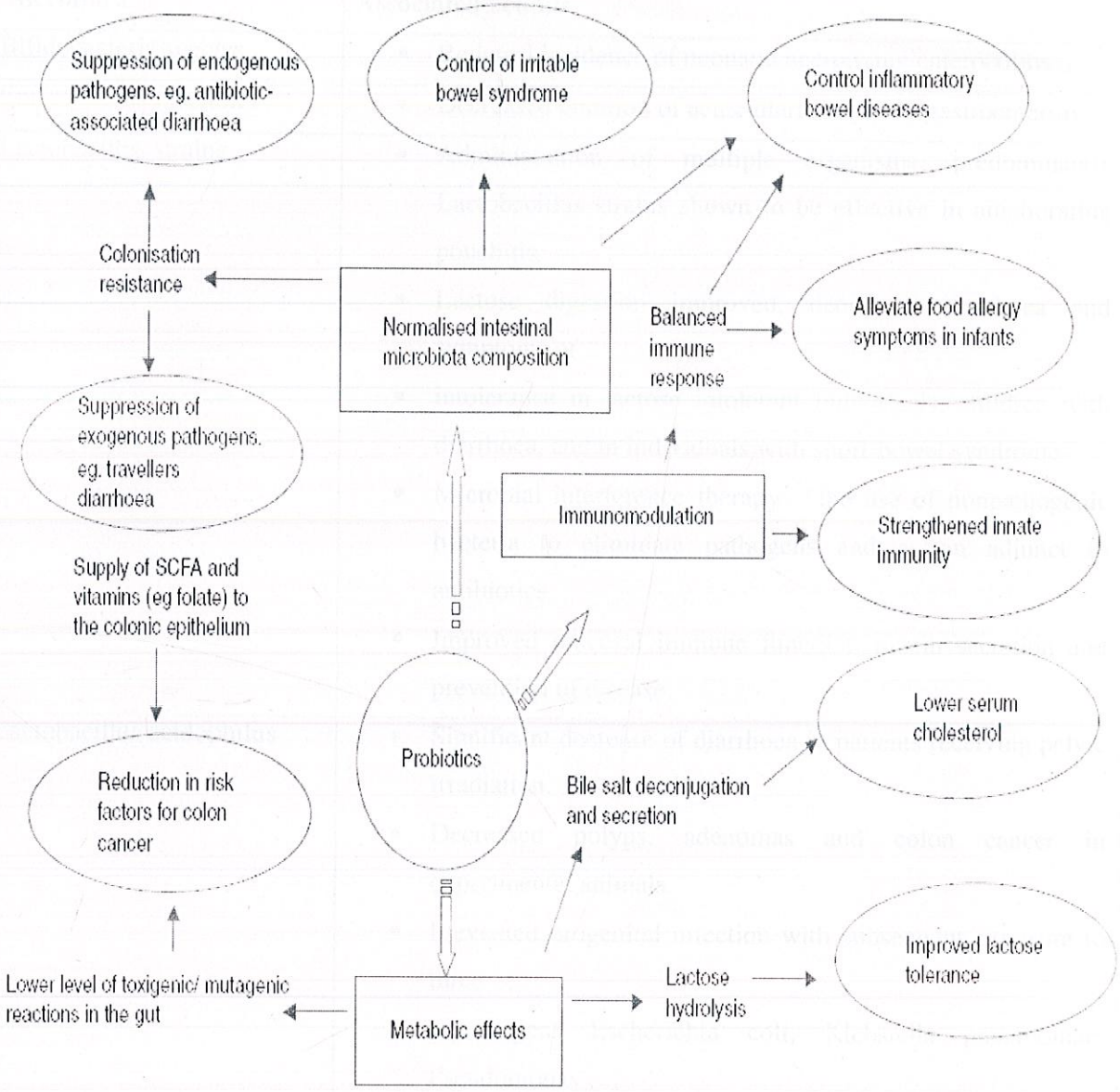


Table – 1.2

Various special therapeutic or prophylactic properties of specific probiotics (Parvez S, et.al 2006)

Microflora	**Associated actions**
Bifidobacteria species	• Reduced incidence of neonatal necrotizing enterocolitis
Enterococcus faecium	• Decreased duration of acute diarrhoea from gastroenteritis
Lactobacillus strains	• Administration of multiple organisms, predominantly Lactobacillus strains shown to be effective in ameliorating pouchitis • Lactose digestion improved, decreased diarrhoea and symptoms of • intolerance in lactose intolerant individuals, children with diarrhoea, and in individuals with short-bowel syndrome • Microbial interference therapy – the use of nonpathogenic bacteria to eliminate pathogens and as an adjunct to antibiotics • Improved mucosal immune function, mucin secretion and prevention of disease
Lactobacillus acidophilus	• Significant decrease of diarrhoea in patients receiving pelvic irradiation. • Decreased polyps, adenomas and colon cancer in experimental animals. • Prevented urogenital infection with subsequent exposure to three • ropathogens Escherichia coli, Klebsiella pneumoniae, Pseudomonas • aeruginosa • Lowered serum cholesterol levels
Lactobacillus plantarum	• Reduced incidence of diarrhoea in daycare centres when administered to only half of the children

	• Especially effective in reducing inflammation in inflammatory bowel; e.g., enterocolitis in rats, small bowel bacterial overgrowth in children, pouchitis • Reduced pain and constipation of irritable bowel syndrome • Reduced bloating, flatulence, and pain in irritable bowel syndrome in controlled trial. • Positive effect on immunity in HIV+ children
Lactobacillus reuteri	• Shortened the duration of acute gastroenteritis. • Shortened acute diarrhea.
Lactobacillus rhamnosus	• Enhanced cellular immunity in healthy adults in controlled trial
Lactobacillus salivarius	• Suppressed and eradicated Helicobacter pylori in tissue cultures and animal models by lactic acid secretion
Bacteroides species	• Chronic colitis, gastritis, arthritis (increased bacterial urease activity in chronic juvenile arthritis)
Saccharomyces boulardii (yeast)	• Reduced recurrence of Clostridium difficile diarrhea. • Effects on C. difficile and Klebsiella oxytoca resulted in decreased risk and/or shortened duration of antibiotic-associated diarrhea. • Shortened the duration of acute gastroenteritis. • Decreased only functional diarrhoea, but not any other symptoms of irritable bowel syndrome

Table - 1.3

The most commonly used species of lactic acid bacteria in probiotic preparations (Parvez S, et.al 2006)

Lactobacillus sp.	Bifidobacterium sp.	Enterococcus sp.	Streptococcus sp.
L. acidophilus	B. bifidum	Ent. faecalis	S. cremoris
L. casei	B. adolescentis	Ent. faecium	S. salivarius
L. delbrueckii ssp. (bulgaricus)	B. animalis		S. diacetylactis
L. cellobiosus	B. infantis		S. intermedius
L. curvatus	B. thermophilum		
L. fermentum	B. longum		
L. lactis			
L. plantarum			
L. reuteri			
L. brevis			

Table - 1.4

1.9.2 Probiotic carriers

Yogurt and fermented milks are considered as the main vehicle for probiotic delivery. Usually yogurt is prepared by allowing milk to ferment by specific pure cultures of lactic acid bacteria (S. thermophilus and L. bulgaricus cultures). Increasingly yogurts have been prepared with probiotic micro-organisms with varying viability over a range of shelf lives. Plain-yogurts demonstrated significant ability in retaining a higher level of L. acidophilus over the shelf life compared to yogurts containing mixed berry or passion fruits. However, interestingly, yogurts containing mango or strawberry contained higher level of L. acidophilus than the plain-yogurts demonstrating the effect of different properties of various fruit mixtures such as pH on the viability of probiotics in yogurt (Adams M.C, et al. 2009).

Ice cream and frozen products as probiotic carrier food and effect on efficacy Ice cream and frozen dairy deserts demonstrated great potential for use as vehicles for probiotic cultures. Ice cream is considered favourably as a probiotic carrier due to the lower storage temperature and less risk of temperature abuse during frozen storage which leads to higher viability of probiotics at the time of consumption (Adams M.C, et al. 2009).

Cheese and chocolate products as probiotic carrier foods and effect on efficacy Cheese is a versatile food product, appealing to many palates and is suitable for all age groups and provides a valuable alternative to yogurt and fermented milk as a vehicle in probiotic delivery. Consumption of cheese has increased in many countries during past decade, providing additional advantage of use of cheese products in probiotic delivery. Furthermore, aerated dairy deserts such as chocolate mousse have also shown a great market potential and potential as probiotic delivering agents (Adams M.C, et al. 2009).

Beverages, cereals and vegetable products and their effect on probiotic efficacy Although most of the current probiotic foods are mainly dairy based, there is a growing interest in the development of non-dairy probiotic products due to problems such as lactose intolerance in

many people and the unfavourable cholesterol content of fermented dairy products. In addition there is an increasing demand for vegetarian probiotic products. This has led to development of probiotic products from various food metrics including fruits, vegetables, legumes and cereal products (Adams M.C, et al. 2009).

Beneficial effects of prebiotics on probiotic bacteria in foods. (Adams M.C, et al. 2009).

Food	Prebiotics	Probiotics	Effect
Yogurt	Hi-maize/resistant	L. acidophilus	Increase Growth and viability
	Starch	L. casei	
	Inulin	L. acidophilus L.casei L. rhamnosus L. reuteri Bifidobaceterium	Increase Growth and viability
	Fructooligosaccharides	L. acidophilus L.casei L. rhamnosus Bifidobaceterium B. animalis B. longum	Increase Viability and fatty acid production
Fermented milk	Polydextose	L. acidophilus L. rhamnosus B. animalis subsp.Lactis	Increase Growth, viability and fatty acid Production
	Oligofructose	L. acidophilus L. rhamnosus B. animalis subsp. lactis	Increase Growth, viability and fatty acid production
Ice cream	Inulin	L. acidophilus B. lactis	Increase Viability

Table - 1.4

CHAPTER 2

MATERIALS AND METHOD

2.1 MATERIALS

1. CHANA(*Cicer arietinum*)

The chickpea (*Cicer arietinum*) (also garbanzo bean, chana (north India), Indian pea, ceci bean, Bengal gram) is an edible legume of the family Fabaceae, subfamily Faboideae. Chickpeas are high in protein and one of the earliest cultivated vegetables; 7,500-year-old remains have been found in the Middle East.[1]

Chickpeas are a helpful source of zinc, folate and protein.[12][13] They are also very high in dietary fiber and hence a healthy source of carbohydrates for persons with insulin sensitivity or diabetes. Chickpeas are low in fat and most of this is polyunsaturated. Nutrient profile of desi chana (the smaller variety) is different, especially the fibre content which is much higher than the light coloured variety. One hundred grams of mature boiled chickpeas contains 164 calories, 2.6 grams of fat (of which only 0.27 grams is saturated), 7.6 grams of dietary fiber and 8.9 grams of protein. Chickpeas also provide dietary phosphorus (49–53 mg/100 g), with some sources citing the garbanzo's content as about the same as yogurt and close to milk.[*citation needed*] According to the International Crops Research Institute for the Semi-Arid Tropics chickpea seeds contain on average:

- 23% protein
- 64% total carbohydrates (47% starch, 6% soluble sugar)
- 5% fat
- 6% crude fiber
- 3% ash

There is also a high reported mineral content:

- phosphorus (340 mg/100 g)
- calcium
- magnesium (140 mg/100g)
- iron (7 mg/100 g)
- zinc (3 mg/100 g)

Recent studies by government agencies have also shown that they can assist in lowering of cholesterol in the bloodstream

2. MOONG *(Vigna radiata)*

The Mung bean, also known as green bean, choroko (in Swahili), mung, mongo, moong, moog (whole) or moog dal (split) (in Bengali , Marathi), mash bean, munggo or monggo, green gram, golden gram, and green soy, is the seed of *Vigna radiata*,. The split bean is known as pesara (Telugu), which is green with the husk, and yellow when dehusked. The beans are small, ovoid in shape, and green in color. The English word "mung" derives from the Hindi: मूँग mung. The mung bean is one of many species recently moved from the genus Phaseolus to Vigna, and is still often seen cited as Phaseolus aureus or Phaseolus radiatus. These variations of nomenclature have been used regarding the same plant species.

Nutritional value per 100 g (3.5 oz)

- Energy 441 kJ (105 kcal)
- Carbohydrates 19.15 g –
- Sugars 2.00 g –
- Dietary fiber 7.6 g

- Fat 0.38 g
- Protein 7.02 g
- Vitamin C 1.0 mg (2%)
- Calcium 27 mg (3%)
- Magnesium 0.298 mg (0%)
- Phosphorus 99 mg (14%)
- Potassium 266 mg (6%)
- Sodium 2 mg (0%)

3. MASOOR(*Lens culinaris*)

The lentil (*Lens culinaris*) is a type of pulse. It is a bushy annual plant of the legume family, grown for its lens-shaped seeds. It is about 40 centimetres (16 in) tall and the seeds grow in pods, usually with two seeds in each.

Lentils contain high levels of proteins, including the essential amino acids isoleucine and lysine, and are an essential source of inexpensive protein in many parts of the world for those who adhere to a vegetarian diet. Lentils are deficient in two essential amino acids, methionine and cysteine. However, sprouted lentils contain sufficient levels of all essential amino acids, including methionine and cysteine.

Apart from a high level of proteins, lentils also contain dietary fiber, folate, vitamin B_1, and minerals. Red (or pink) lentils contain a lower concentration of fiber than green lentils (11% rather than 31%). Health magazine has selected lentils as one of the five healthiest foods. Lentils are often mixed with grains, such as rice, which results in a complete protein dish.

Lentils also have "anti-nutritional factors" such as trypsin inhibitors and relatively high phytate content. Trypsin is an enzyme involved in digestion and phytates reduce the bioavailability of dietary minerals.[9] The phytates can be reduced by soaking the lentils in warm water overnight.

Nutritional value per 100 g (3.5 oz)

- Energy 1,477 kJ (353 kcal)
- Carbohydrates 60 g –
- Sugars 2 g –
- Dietary fiber 31 g
- Fat 1 g
- Protein 26 g
- Thiamine (Vit. B_1) 0.87 mg
- Folate (Vit. B_9) 479 µg
- Iron 7.5 mg

2.2 METHODS

1. EXTRACTION

A. CHANA(*Cicer arietinum*)

BOILED EXTRACT

- 20gm of chana was weighed and washed to remove the foreign unwanted particles.
- 500 ml of distilled water was taken in a beaker.
- The chana was boiled in distilled water for 1hr.
- The supernatant was separated from the precipitate and the final volume was measured.
- The extract was centrifuged at 5000 rpm for 20 minutes to remove the suspended particles.
- This extract was autoclaved, sealed and stored in cold at 4 C.

GERMINATED EXTRACT

- 20gm of chana was weighed and washed to remove the foreign unwanted particles.
- The chana were covered with muslin cloth, soaked in water and kept for 72 hours at room temperature and frequent resoaking with water is done.
- The germinated chana were weighed and kept in oven at 60^{o} C for 24 hours.
- The dried chana were then powdered using grinder.
- 500ml of distilled water was take in a beaker.
- The chana were boiled in distilled water for 1 hr.

- The supernatant was separated from the precipitate and the final volume was measured.
- The extract was centrifuged at 5000 rpm for 20 minutes to remove the suspended particles.
- This extract was autoclaved, sealed and stored in cold at 4 ^{o}C.

GRINDED EXTRACT

- 20 gm of chana was weighed and washed to remove the foreign unwanted particles.
- The chana was then powdered using grinder.
- 500 ml of distilled water was taken in a beaker.
- The chana was boiled in distilled water for 1hr.
- The supernatant was separated from the precipitate and the final volume was measured.
- The extract was centrifuged at 5000 rpm for 20 minutes to remove the suspended particles.
- This extract was autoclaved, sealed and stored in cold at 4 ^{o}C.

B. MOONG *(Vigna radiata)*

BOILED EXTRACT

- 20gm of moong was weighed and washed to remove the foreign unwanted particles.
- 500 ml of distilled water was taken in a beaker.
- The moong was boiled in distilled water for 1hr.
- The supernatant was separated from the precipitate and the final volume was measured.

- The extract was centrifuged at 5000 rpm for 20 minutes to remove the suspended particles.
- This extract was autoclaved, sealed and stored in cold at 4 C.

GERMINATED EXTRACT

- 20gm of moong was weighed and washed to remove the foreign unwanted particles.
- The moong were covered with muslin cloth, soaked in water and kept for 72 hours at room temperature and frequent resoaking with water is done.
- The germinated moong were weighed and kept in oven at 60° C for 24 hours.
- The dried moong were then powdered using grinder.
- 500ml of distilled water was take in a beaker.
- The moong were boiled in distilled water for 1 hr.
- The supernatant was separated from the precipitate and the final volume was measured.
- The extract was centrifuged at 5000 rpm for 20 minutes to remove the suspended particles.
- This extract was autoclaved, sealed and stored in cold at 4 °C.

GRINDED EXTRACT

- 20 gm of moong was weighed and washed to remove the foreign unwanted particles.
- The moong was then powdered using grinder.
- 500 ml of distilled water was taken in a beaker.
- The moong was boiled in distilled water for 1hr.

- The supernatant was separated from the precipitate and the final volume was measured.
- The extract was centrifuged at 5000 rpm for 20 minutes to remove the suspended particles.
- This extract was autoclaved, sealed and stored in cold at 4 °C.

C. **MASOOR(*Lens culinaris*)**

BOILED EXTRACT

- 20gm of masoor was weighed and washed to remove the foreign unwanted particles.
- 500 ml of distilled water was taken in a beaker.
- The masoor was boiled in distilled water for 1hr.
- The supernatant was separated from the precipitate and the final volume was measured.
- The extract was centrifuged at 5000 rpm for 20 minutes to remove the suspended particles.
- This extract was autoclaved, sealed and stored in cold at 4 C.

GERMINATED EXTRACT

- 20gm of masoor was weighed and washed to remove the foreign unwanted particles.
- The masoor were covered with muslin cloth, soaked in water and kept for 72 hours at room temperature and frequent resoaking with water is done.
- The germinated masoor were weighed and kept in oven at 60° C for 24 hours.

- The dried masoor were then powdered using grinder.
- 500ml of distilled water was take in a beaker.
- The masoor were boiled in distilled water for 1 hr.
- The supernatant was separated from the precipitate and the final volume was measured.
- The extract was centrifuged at 5000 rpm for 20 minutes to remove the suspended particles.
- This extract was autoclaved, sealed and stored in cold at 4 °C.

GRINDED EXTRACT

- 20 gm of masoor was weighed and washed to remove the foreign unwanted particles.
- The masoor was then powdered using grinder.
- 500 ml of distilled water was taken in a beaker.
- The masoor was boiled in distilled water for 1hr.
- The supernatant was separated from the precipitate and the final volume was measured.
- The extract was centrifuged at 5000 rpm for 20 minutes to remove the suspended particles.
- This extract was autoclaved, sealed and stored in cold at 4 °C.

2. TOTAL CARBOHYDRATE CONTENT BY ANTHRONE METHOD

Anthrone Reagent

It was freshly prepared by adding 200 mg of anthrone reagent in 100 ml of distilled water.

Standard Glucose Solution(GSS)

It was prepared by adding 10 mg of glucose to 100 ml of distilled water.

PROCEDURE

- Standard differential concentration of glucose was prepared in a test tube by adding 200, 400, 600, 800 µl of GSS in 800, 600, 400, 200 µl of distilled water respectively.
- 4 ml of anthrone reagent was added to each test tube and mixed well.
- The test tubes were covered with foil and a small hole was made to let the air pass.
- The test tubes were kept in a boiling water bath for 10 minutes.
- The test tubes are cooled to room temperature.
- OD was measured at 620 nm.

3 PROTEIN ESTIMATION.

Although Lowry method is not good enough compared to Bradford and Bicinchoninic Acid method for protein quantitation, but it is still widely used due to its acceptability to estimate protein in almost all circumstances in which protein mixtures or crude extracts are involved. Lowry method is based on the conversion of Cu^{2+} to Cu^{+} under alkaline conditions. The reactions result in a strong blue colour, which depends partly on the

tyrosine and tryptophan content. Sensitivity of the method is down to about 0.01 mg of protein /mL, and is best used on solutions with concentrations in the range 0.01-1.0 mg/mL of protein. If the sample contains protein less than 0.01, then alternative method i.e Bradford method (it can be used for measuring between 1 and 10 micrograms of protein, for microassay) and Bicinchoninic Acid method (it can be used for measuring between 0.5 and 10 micrograms of protein/mL, for microassay).

Material

1. Complex-forming reagent: Prepared immediately before use by mixing the following stock solutions in the proportion 100:1:1 (by vol), respectively:

Solution A: 2% (w/v) Na2CO3 in distilled water.

Solution B: 1% (w/v) CuSO4•5H2O in distilled water.

Solution C: 2% (w/v) sodium potassium tartrate in distilled water.

2. 2 N NaOH.

3. Folin reagent (commercially available): Used at 1 N concentration.

4. Standards: We used a stock solution of standard protein (e.g., bovine serum albumin fraction V) containing 2 mg/mL protein in distilled water, stored frozen at –20°C. Prepared standards by diluting the stock solution with distilled water.

The Method :

1.To 0.1 mL of sample or standard, 0.1 mL of 2 N NaOH was added. Hydrolyzed at 100°C for 10 min in a heating block or boiling water bath.

2. The hydrolysate was cooled to room temperature and 1 mL of freshly mixed complex-forming reagent was added. Solution was allowed to stand at room temperature for 10 min.

- The reaction was very pH dependent, and it was therefore important to maintain the pH between 10 and 10.5.

3. 0.1 mL of Folin reagent was added , using a vortex mixer, and the mixture was allowed to stand at room temperature for 30–60 minutes.

4. The absorbance was read at 650 nm if the protein concentration was below 500 micrograms/mL

and at 550 nm if the protein concentration was between 100 and 2000 micrograms/mL.

5.A standard curve of absorbance was plotted as a function of initial protein concentration and used to determine the unknown protein concentrations.

- The assay is not linear at higher concentrations. So we ensured that we are analyzing your sample on the linear portion of the calibration curve.
- Triplication was done in order to reduce experimental errors.

One disadvantage of the Lowry method is the fact that a range of substances interferes with this assay, including buffers, drugs, nucleic acids, and sugars. In many cases, the effects of these agents can be minimized by diluting them out, assuming that the protein concentration is sufficiently high to still be detected after dilution.

We can also increase Lowry method's sensitivity by doing these two following things:

1.If the Folin reagent is added in two portions, vortex-mixing between each addition, a 20% increase in sensitivity is achieved.

2.The addition of dithiothreitol 3 min after the addition of the Folin reagent increases the sensitivity by 50%.

4 Evaluation of suitability of the pulse extract for the growth of Lactobacillus sp. by calculate the CFU/ml (Colony forming unit).

Strain used

Lactobacillus plantarum

Lactobacillus rhamnosus

Maintenance of Culture

- MRS medium (de Man Rogosa Sharpe medium).
- The culture will be grown at 37^0C for 24 hrs in MRS medium and used as inoculums in juice.
- Media is sterilized by Autoclave at 121^0C at 15 psi.

Fermentation conditions

- All samples of pulses will be inoculated with 24 hrs culture and incubated at 40^0C , 140rpm for 48 hours.

Cell Viability

Viable cell count (CFU/ml) will be determined by standard plate count method.

CHAPTER 3

RESULTS

3.1 Standardization and Estimation of Carbohydrate content by using anthrone method.

Experiment is repeated 2 times. All readings are taken in duplets.

X-axis = concentration (µg/ml) ; Y-axis = OD at 620nm

Standard Graph

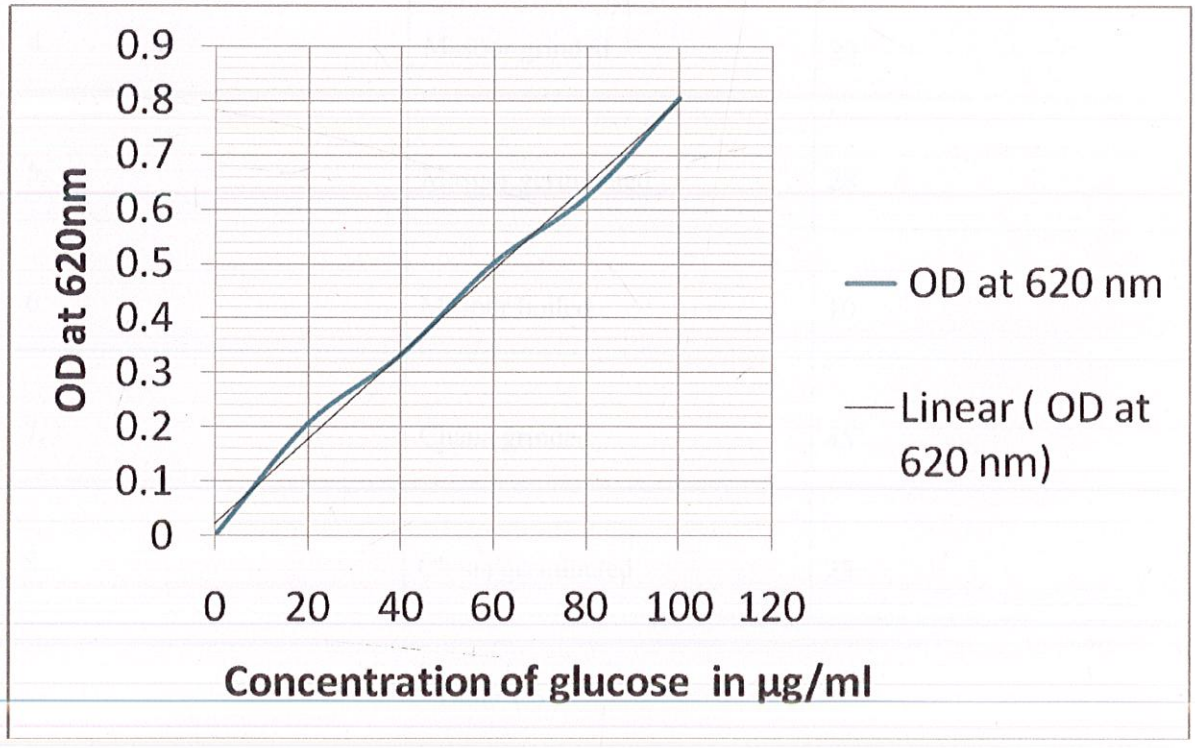


Figure 8

Estimation of concentration of Total carbohydrate Content in different samples of pulses.

Experiment is repeated 2 times. All readings are taken in duplets.

S.No	Sample	Concentration (mg/mL)
1.	Moong grinded	19
2.	Moong germinated	21
3.	Moong boiled	12
4.	Masoor grinded	23
5.	Masoor germinated	28
6.	Masoor boiled	10
7.	Chana grinded	45
8.	Chana germinated	25

Table 1.5

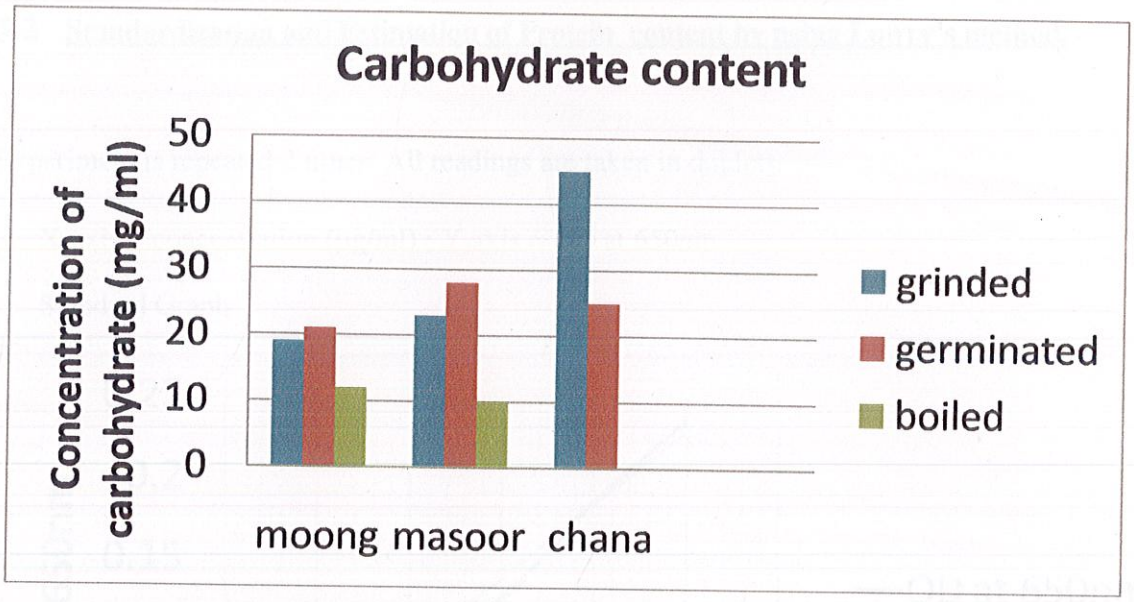

Carbohydrate content
Concentration of carbohydrate (mg/ml)
50
40
30
20
10
0
moong masoor chana
grinded
germinated
boiled

Figure 9

3.2 Standardization and Estimation of Protein content by using Lowry's method.

Experiment is repeated 2 times. All readings are taken in duplets.

X-axis = concentration (µg/ml) ; Y-axis = OD at 650nm

Standard Graph

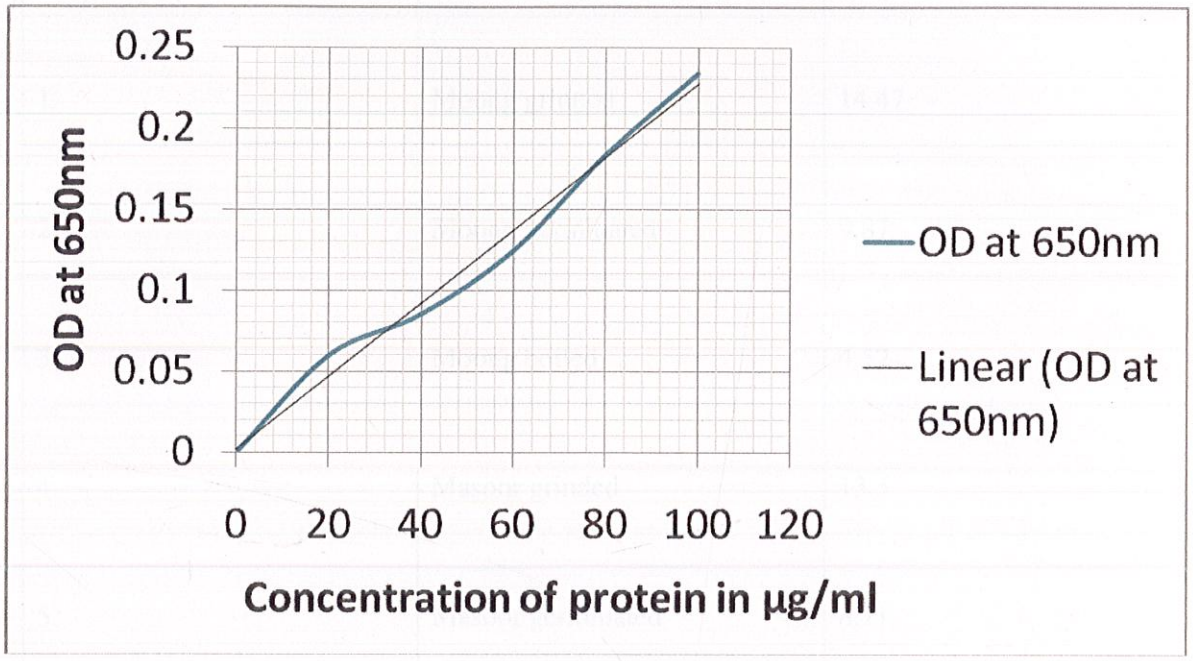


Figure 10

Estimation of concentration of Protein Content in different samples of pulses.

Experiment is repeated 2 times. All readings are taken in duplets.

S.No	Sample	Concentration (mg/mL)
1.	Moong grinded	14.47
2.	Moong germinated	9.87
3.	Moong boiled	4.52
4.	Masoor grinded	13.5
5.	Masoor germinated	8.71
6.	Masoor boiled	3.93
7.	Chana grinded	6.96
8.	Chana germinated	6.28

Table- 1.6

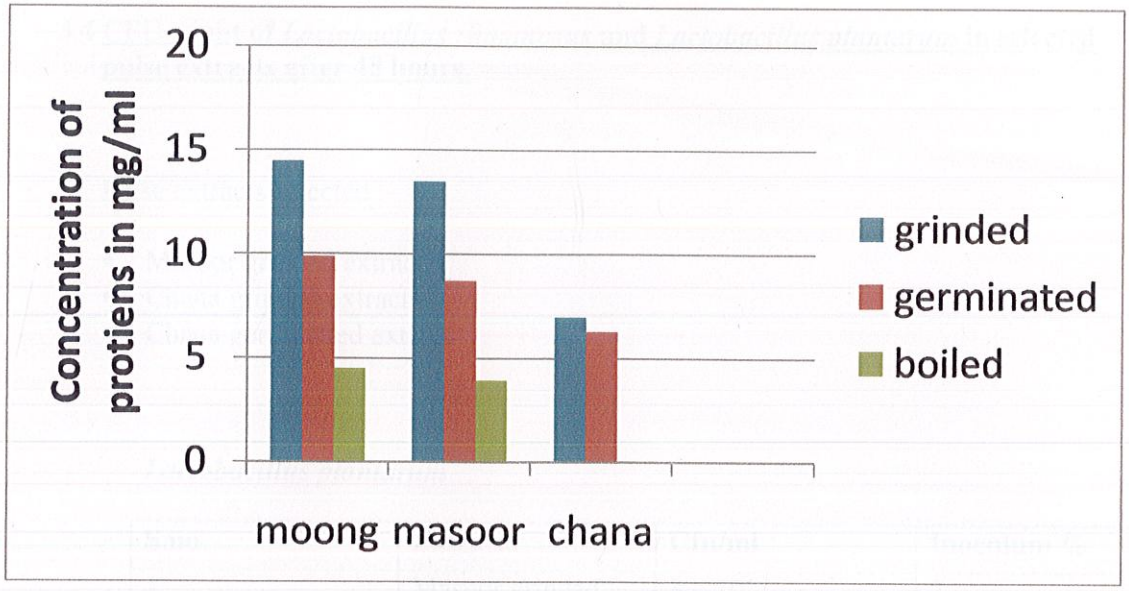

Concentration of protiens in mg/ml
20
15
10
5
0
moong masoor chana
grinded
germinated
boiled

Figure 11

3.4 CFU count of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* in selected pulse extracts after 48 hours.

Pulse extracts selected :-

- Masoor grinded extract
- Chana grinded extract
- Chana germinated extract

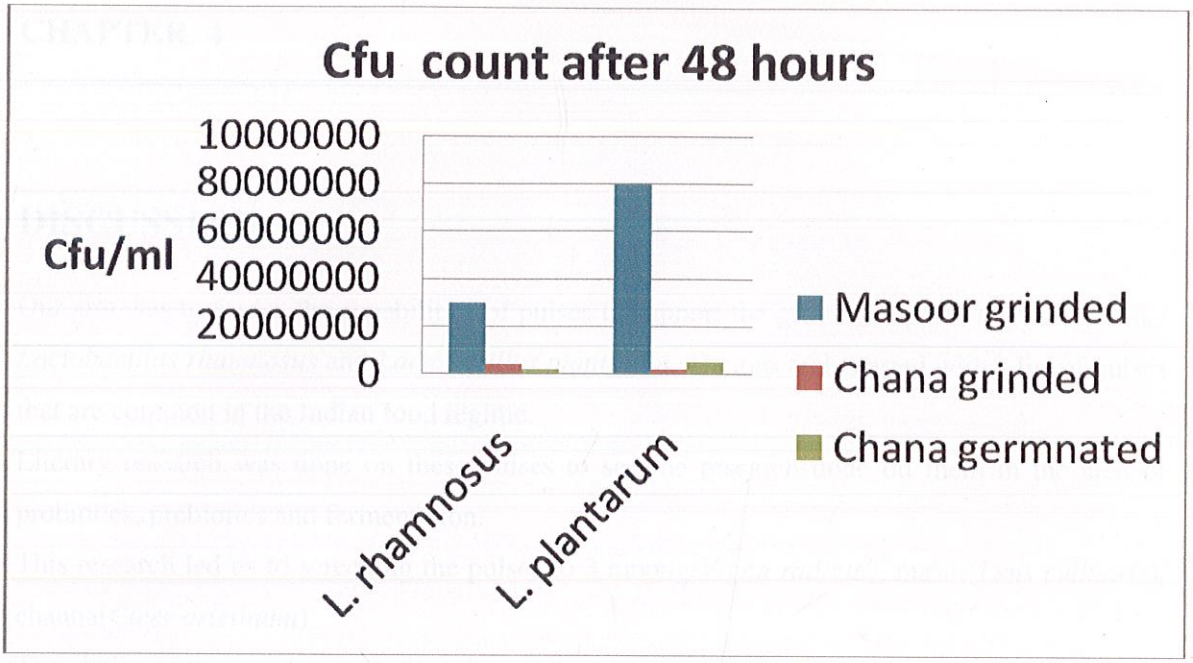
Lactobacillus plantarum

S.no	Extracts	Cfu/ml	Inoculum %
1	Masoor grinded	8×10^7	2
2	Chana grinded	2×10^6	2
3	Chana germinated	5×10^6	2

Lactobacillus rhamnosus

S.no	Extracts	Cfu/ml	Inoculum %
1	Masoor grinded	3×10^7	2
2	Chana grinded	4×10^6	2
3	Chana germinated	2×10^6	2

Table- 1.7


Cfu count after 48 hours
Cfu/ml
10000000
80000000
60000000
40000000
20000000
0
L. rhamnosus
L. plantarum
Masoor grinded
Chana grinded
Chana germnated

CHAPTER 4

DISCUSSION

Our aim was to assess the capabilities of pulses to support the growth of probiotic bacteria like *Lactobacillus rhamnosus* and *Lactobacillus plantarum*. The research started with a list of pulses that are common in the Indian food regime.

Literary research was done on these pulses to see the research done on them in the area of probiotics, prebiotics and fermentation.

This research led us to screening the pulses to 3 moong*(Vigna radiata)*, masur(*Lens culinaris*), channa(*Cicer arietinum*).

The choice of these pulses were based on te fcat that these pulses though very popular in Indian food regime have remained underexplored as far as carrier for probiotic bacteria is concerned..

The next step was to optimize the extraction method in order to get the maximum nutrients present in these pulses and available for the bacteria. We started with three types of extraction processes to see which proves to be the most efficient. Our extraction processes included a boiled extract, germinated an extract and a grinded extract. All the three extraction processes were aimed at achieving the same goal, i.e, extraction of maximum possible nutrients from the pulses. The boiled extract followed the basis of normal cooking methods of pulses. The germinated extract gave time for the plant metabolism to start and then the nutrients were extracted.

The grinded extract aimed simply to get the pulse into powdered form so that every component gets out. These extracts had to be checked for their nutritional characteristics.

We started with carbohydrate estimation following the anthrone method. The results showed some interesting characteristics. The effect of germination was different in case of different pulses. In the case of masoor the amount of carbohydrates was more in case of germinated extract as compared to grinded extract, but the scenario was opposite for chana. Overall though

grinded chana extract had maximum carbohydrate concentration of 45mg/ml followed by germinated masoor extract having carbohydrate concentration of 28mg/ml.

Followed by carbohydrate estimation was protein estimation using lowry's method. The pulse with the highest protein content was masoor in its grinded extract.

The results of carbohydrate and protein estimation showed us that boiled extract almost always had less nutrients then their respective counterparts.

Following up with these results, three extracts were selected for probiotic testing by setting up fermentations with *Lactobacillus plantarum* and *Lactobacillus rhamnosus* strains.

Masoor grinded extract, chana grinded extract and chana germinated extracts were selected.

Unfortified pulses extracts showed promising growth of *Lactobacillus plantarum* and *Lactobacillus rhamnosus.* This growth can be further enhanced by performing fortification studies on the extracts. The innoculum percent which was kept at 2% for the above study could be increased to get the final cfu/ml in the range of 10^9 - 10^{10}

CHAPTER 5

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RESUME

NAME: Kanishka Sinhal

CGPA : 6.0

Industrial Training : Undertook training in R.N Herbal Pvt Limited and worked on "Production of Herbal Extract for manufacturing of Ayurvedic cool Oil".

Area of Interest : Food Biotechnology, Plant Tissue Culture.

Hobbies : Travelling, Driving.

NAME: Rushil Bansal

CGPA : 5.5

Industrial Training : Have studied about Isolation, identification and handling of various environmental organisms in WOCKHARDT.

Area of Interest : Studying about probiotics and its health benefits to humans and Programming in computers.

Hobbies : Playing cricket, Snooker and Reading books.

NAME: Varun Dhir

CGPA : 5.6

Industrial Training : Undertook industrial training on "Study of various species of Algae and determining their biological characteristics" at Indian Agricultural Research Institute,New Delhi

Area of Interest : Genetic Engineering

Hobbies : Playing drums, Listening to Music, Travelling.