“PRODUCTION OF PROBIOTIC BEVERAGE FROM FINGER MILLETS BY USING *Lactobacillus* spp.”

A

PROJECT REPORT

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Probiotics from Finger Millet

"Dissemination of education for Knowledge, Science and Culture"

-Shikshanmaharshi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLEGE KOLHAPUR
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LABORATORY CERTIFICATE

This is to certify that Ms. Sonali Manohar Gawade studying in M.SC II Microbiology at Vivekanand college, Kolhapur. She have sincerely completed Project work entitled as **"PRODUCTION OF PROBIOTIC BEVERAGE FROM FINGER MILLETS BY USING *Lactobacillus* SPP."** prescribed by Vivekanand College, Kolhapur (Empowered autonomous) during academic year 2023-2024.

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Place: Kolhapur

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CHAPTER-1

INTRODUCTION

INTRODUCTION

Probiotics are living microorganisms, that can have beneficial effects in human body .Trillions of microorganism are present in every human body. These microbes helps by supporting our body function and improve our health. All microbes that live with us are not helpful. Somme microbes can be harmful. But beneficial microbes like Probiotics helps to control the potentially harmful types,

Probiotics provides health benefits to host when administered in optimum amount. The strains of *Bifidobacterium* and *Lactobacillus* are predominant groups of probiotic organisms. These are included in many food and dietary supplements. Due to prove beneficial effects of Probiotics on human health the food industry has become increasingly interested in these types of microorganisms.

Probiotic microorganisms are also good for our digestive system. But our body is full of bacteria both good and bad probiotic are often called “good” or “helpful ” bacteria because they help to keep our gut healthy.

In food you can found Probiotics as supplements for e.g., Yoghurt, Sauerkraut, Kefir, Kimchi, Kombucha. In disease we take antibiotics that affects our body normal microflora which may causes loss of “good” bacteria in our body, Probiotics can helps replace them, Probiotics helps us to maintain good microflora to keep our body working.

Many types of bacteria are classified as probiotic includes *Saccharomyces, Streptococcus, Enterococcus, Eishcherichia* and *Bacillus*. They all have different benefit, but most are come from Spp. *Lactobacillus* and *Bifidobacterium*

Lactobacillus – It is the most common probiotic organism, it is found in yogurt and other fermented food, Probiotic food containing *lactobacillus* is beneficial for lactose intolerant peoples.

Bifidobacterium - It is found in dairy products.

For population, probiotic are food and health products that contribute live beneficial microbes in our gut and elsewhere , They prevent and treat dysbiosis and imbalance on a deficit of beneficial microbes in our body. To strength this communities, microbes take up

residence on or in our body. Healthcare provides might recommend probiotic to help in bringing our microbiome back to balance Due to heavy antibiotic dose, sometime, normal flora is disturbed and may also weaken our microbiome. In this case healthcare provides might suggest the use of probiotic to rebuild it.

Some people to maintain their general wellness, takes daily probiotic as supplement. A healthy gut microbiome can boost your overall immunity, reduce inflammation and helps keep our bowel movement regular. Our nowadays lifestyle can affect our gut microbiome but Probiotics are one way to help to restore and rebuild them.

Probiotics improve our body's resistance against pathogenic microorganism by providing fatty acids, vitamins, and other vital nutrients. Number of probiotics are present in fermented food, that produces nutrients like vitamin and improve digestion. Probiotic organisms prevent growth of spoilage causing organism in food. They can prevent or treat diarrhea. They prevent tooth decay and can control, some type of diabetes and also control or reduces "bad" type of cholesterol. Much research proved that addition of Probiotic to food leads to several health benefit including the reduction of level of serum cholesterol, and can enhancement of immune system and also improve is gastrointestinal function, probiotic organisms also play crucial role in suppression of diarrhea in young children and also lowers the risk of colon cancer [Berner and Donnel 1998] Traditionally dairy products have been the vehicle for the probiotic, which involves lactic acid bacteria. According to a number of scientific and popular sources, probiotics were initially used by humans around 2000 B.C., when they figured out how to prolong the shelf life of milk. The milk was actually fermented into dairy products by the first food manufacturers.

In 1953, German scientist Werner Kollath coined the term "probiotic" (derived from the Latin pro and the Greek literally meaning "for life") to refer to "active substance that are required for a healthy development of life. "Lilly and Stillwell used the term in 1965, although in a different sense, to express."Substances secreted by one organization that promote another's growth."Further in 1992, probiotics were defined as "a live microbial feed additive which improves the intestinal microbial balance of the host animal, hence benefiting it."

Beginning in the early 1900s, Russian scientist Elie Metchnikoff, who would go on to win the Nobel Prize, conducted groundbreaking research on probiotics at the Pasteur Institute in Paris. However, Louis Pasteur discovered the microbes in charge of the fermentation process. Metchnikoff initially tried to determine whether these bacteria would have an impact on human health.

Health benefits:

- **Impact on Dental Health:**

The development of probiotics for pertinent therapeutic health advantages has led to a focus on the use of probiotics for applications outside of the gut. Probiotics' primary goal in oral health applications is to diagnose the cariogenic mouth, much like in non-gut uses like dental care.
When taken once or twice daily, the probiotic has been found to be a beneficial addition to root.

Research has shown that probiotics can be utilized in biological plaque control techniques as an alternative to chemical plaque treatment; the mechanism of action of this treatment includes immune function alterations and antibacterial action. (Palanisamy Devi,2014)

- **Lactose intolerance**:

A genetically determined beta galactosidase deficit that prevents lactose from being hydrolyzed into the monosaccharides glucose and galactose is known as lactose intolerance. Osmotic diarrhea results from the breakdown of undigested lactose by bacterial enzymes in the large intestine. Beta galactosidase deficiency can be caused by acquired, typically reversible conditions such as short bowel syndrome, rotavirus infection, which damages mucosa, and pelvic radiation therapy. Those who are lactose intolerant have flatulence, diarrhea, and discomfort in the abdomen after consuming milk or milk products. While traditional yoghurt recipes, making use of *S. thermophilus* and *L. delbrueckii* Spp. (Palanisamy Devi,2014)

- **Allergies and Probiotics:**

According to recent research, early life exposure to bacteria may protect against allergies. In this regard, probiotics may offer the safe alternative microbial stimulation that newborns' growing immune systems require. Simultaneously, they enhance the mucosal barrier function, a characteristic thought to have a role in regulating the allergic reaction. Measurements of the gut microbiota's quantitative and qualitative variations between allergic and healthy children and infants the former showing colonization by a more adult like type of microflora support the significance of intestinal microbiota in allergy.

- **Impact on the Immune system**

Probiotics have an impact on human immunity. Among their many other positive health benefits, they affect epithelial barrier function and proliferation. Probiotics and their effector molecules affect the gut flora. The amount of probiotics in a regular meal could vary from 2 to 20 g per day, depending on the ingredient. the understanding of prebiotics' ability to promote health. which enhance reduce illness risk & A uses in treatment**.**

- **Persistent Kidney Disease**

Probiotic related worries addition increased as more chronic renal disease patients were recorded. The primary objective of administering probiotics during chronic kidney disease is to prevent hemodialysis and kidney transplantation.is solute removal in uremic retention in nephrolithiasis, lactobacilli are given as supplements and aid to avoid stone formation and lower the risk of urolithiasis. These probiotic bacteria attach directly to oxalate molecules, blocking the absorption of the latter via the gut wall.(Palanisamy Devi,2014)

- **Impact on Digestive Health**

Probiotics have been used to enhance gut health for a long time. Probiotics regulate the functions of the intestinal epithelium by maintaining the epithelial barrier, promoting cell survival, increasing the synthesis of antibacterial agents and proteins that shield cells, enhancing protective immune responses, and inhibiting the production of proinflammatory cytokines such as IFN y, TNF a, IL 4, and IL 13. Intestinal function may be affected by pathogenic organisms and their toxins, including enteropathogenic *E. coli, Clostridium perfringens,* and *Vibrio cholerae*. Probiotics have been shown to enhance intestinal epithelial integrity by restoring the balance of the gut microbiota profile, in addition to their direct control over intestinal epithelial cells through probiotics or probiotic derived functional molecules.

- **Body Fat:**

Obesity has emerged as a clinical epidemic because of diminished energy expenditure, increased food consumption, and absorption. Several recent studies have confirmed that human gut bacteria, through effective energy production and nutrient

absorption, play a major role in the development of obesity. Furthermore, it is still recognized that obese people have a more diversified duodenal microbiota than thin people.

- **Mellitus diabetes:**

A serious global health issue, diabetes mellitus is characterized by insufficient insulin secretion and/or action. Type 1 diabetes, also known as insulin dependent diabetes or juvenile onset diabetes, is an autoimmune disease that involves the death of pancreatic beta cells.

The body misuses insulin in type 2 diabetes mellitus, and insulin resistance is frequently present. The gut microbiome is different in diabetics. Bacterial genes, metabolic pathways, and gut microbes were revealed to be significantly correlated in type 2 diabetes patients by the human metagenome broad association study. There was a significant difference in the quantity of *Lactobacillus Spp*. amongst people with diabetes and those without. Levels of glycated hemoglobin and fasting glucose. (Palanisamy Devi,2014)

Millets:

In developing countries millets are the major food. In developing countries the production and consumption of millets is more than 97%, to estimates in 1961 and 2018 the area of land used for millets consumption has decreased worldwide by about 25.71% further more in twentieth century millet productivity increased globally by 36% from 575kg\ha to 900kg\ha

- Millet production in India peaked in the 1980's and the total progressively declined as a result of an increasing reduction in the area that is cultivated, the most millets production in India up to 37.5% of the total global output followed by Sudan and Nigeria

The region where millets are grown is continuing to shrink for an number of reasons, such as shifting millets for other crops and changes in dietary preferences certainly of the infrastructure needed for irrigation and assured profits from significant commercial crops Millets are frequently cultivated in soil that are insufficient to support other crops. Their short growing season, resistance to temperature fluctuation, insufficient nitrogen treatment, and drought tolerance set them apart from other cereal crops.

Millets are all-season, year-round crops that offer a range of security (food, fodder ,nutrition, and environment), making them the agriculture secure crops that are reasonably priced. (Malathi and colleagues , 2016).

The main millets cultivated in India include Sorghum, Pearl millets, Finger millets, Foxtail millets, Tiny millet, Kodu millets, Proso millets, and Barnyard millets Among these finger millet{Eleusine coracana l.} is rich in calcium, and it is almost ten times higher than that of rice or wheat { Malathi et al. 2016}.

It is a member of the family Poaceae. Some of its beneficial components include vitexin, myricetin, quercetin, apigenin, epicatechin, gallocatechin, and epigallocatechin. Possessing antimicrobial, antioxidant, antitumorigenic, and diabetic qualities.

Millets are considered a significant grain globally, yet they are also the least used. Because millet grain is high in nutrients and phenolic compounds that are good for you, it may be used for both food and feed. When choosing millet for food or feed, the kind of millet available matters because fin ger and pearl millet have different nutrient contents and phenolic chemicals. Millets' phenolic qualities include flavonoids, tannins, and phenolic acid all of which are beneficial to human health. Furthermore, when compared to pearl millet, finger millet has an incredibly distinct, plentiful, and varied phenolic profile. Studies have indicated that the phenolic compounds of millet have a strong antioxidant effect.

The term "pseudo millets" refers to grains that are not members of the Poaceae botanical family, which includes "true" grains. Despite this, pseudo millets have similar nutritional qualities.

Millets are a non-glutinous, nonacid forming, and incredibly nutritious food. Millets are rich in fiber and contain a lot of health promoting and nutraceutical qualities. A probiotic food source for the microflora within our inner ecology is millets.

Nutritional Composition :

Among the cereals, millets are special due to their abundance in calcium, dietary fire, proteins, and polyphenols. Millets are a great source of minerals, including calcium, potassium, phosphorus, and magnesium, which contribute significantly to the recommended daily allowance. However, the main ingredients of millets include a sizable number of vital amino acids, specifically methionine and cysteine. Additionally, they contain more fat than sorghum, rice, and maize. Plant nutrition is mostly utilized in the food industry, cereal grains are a key source of dietary nutrients globally, and cereal proteins-including millets-have a limited amount of lysine and tryptophan.

Conversely, ragi is a nutrient-dense powerhouse that aids in weight loss and is used to cure a number of illnesses, including diabetes, brittle bones, osteoporosis, and anemia.

It is a naturally occurring relaxant that eases tension and anxiety. Because of their comparable constitution to wheat, these millets should also contain probiotic molecules such insulin in addition to the arabinoxylan present in

Because millets are known to be a good source of minerals like magnesium, manganese, and phosphorus as well as rich in phytochemicals like phytic acid, which is thought to lower cholesterol, and phylate, which is linked to a lower risk of cancer, there is a huge opportunity to investigate the technological possibilities of using this crop in the food industry to prepare a variety of food products.

Regarding nutrition, finger millet is an excellent source of minerals, fiber, and vitamins, especially calcium. Finger millet has the following composition: around 7% protein, 1%–2% fat, 66% carbs, 1% dietary fiber, and 2% minerals. Of the millets that were chosen, finger millet has the highest carbohydrate content.

Millets are truly super grain They can provide health benefits to consumers & allow farmers more economic stability. Stabilization of millet beverages is important as they have the tendency to delaminate.

The acceptance of millet beverages are day by day increases the millet based beveragehas gluten-free millets are historically and largely consumed in parched and semi-dry regions. Millets also provide nutraceutical benefits in the form of antioxidants Millets are used to make traditional drinks Such as malt, Jandh, pito.

Millets are nutritionally an excellent source. It helps in maintain the cardiovascular health. & reduces acidity problems.

Examples:

(1) pyogurt and Kefir. .

(2) Sauerkraut, Kimchi, pickles

(3) Miso, tamari

(4) Tempeh

(5) Kombucha

***Millets and their nutrimental value.**

Name	Protein (g)	Fiber (g)
Pearl millet	10.6	1.3
Finger millet	7.3	3.6
Foxtail millet	12.3	8
Proso millet	12.5	2.2

***Nutritional health benefits of millets**

Millets are bound to be helpful with the reduction of weight, BMI & high blood pressure.

In India, millet is generally Consumed with legumes which creates mutual supplementation of protein, increases the amino acid content & enhances the overall digestibilityof protein.

***Advantages :-**

- Helps break down and absorb certain nutrients and medications.
- Produces other important nutrients. As by products.
- Helps break down & recycle bile after digestion.
- Helps train your immune system to recognize & eliminate harmful microbes.

***Disadvantages :-**

- Diarrhea – There is strong & Solid evidence that probiotics. Address antibiotic associated diarrhea..
- Another side effect to be aware of is • an upset stomach..
- You may be taking a probiotic to help relieve symptoms such as gas and bloating.

Certain individuals may notice. Headaches when they begin taking probiotics.

- **Are probiotics dangerous?**

Probiotics have a long history of usage that seems safe, especially in healthy individuals. However, there is a dearth of reliable data regarding the frequency and seriousness of adverse effects due to the lack of studies that have examined the safety of probiotics in detail.

Probiotic side effects are more likely to affect those who already have serious illnesses or weakened immune systems. Probiotics should have the possible dangers and benefits carefully examined when prescribed for high-risk populations, such as preterm infants or critically ill hospital patients. Probiotics have been associated with cases of serious or fatal infections in premature newborns; the U.S. Food and Drug Administration (FDA) has alerted medical professionals to this risk. Probiotics may have negative consequences on the body such as infections, the generation of toxic compounds by the microbes, and the spread of antibiotic resistance genes to other microorganisms in the digestive system.

It has been noted that several probiotic products include bacteria not specified on the label. These pollutants may occasionally present significant health hazards.

Beginning in the early 1900s, Russian scientist Elie Metchnikoff, who would go on to win the Nobel Prize, conducted groundbreaking research on probiotics at the Pasteur Institute in Paris. However, Louis Pasteur discovered the microbes in charge of the fermentation process. Metchnikoff initially tried to determine whether these bacteria would have an impact on human health.

CHAPTER- 2

AIMS AND OBJECTIVE

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❖ Probiotics-

The probiotics are foods or supplements that contain live microorganisms which improves the "good bacteria (normal microflora) in the body. The probiotic Bacteria provides many health benefits including digestive health, reduce depression, promote heart health, boost immunity.

❖ Probiotics from finger millets

Millets are exceptional to cereals due to the richness of micronutrients and macronutrients. The finger millets are a good source of fibres, proteins and Carbohydrates. Due to its nutritional richness, it can be used as a substrate for probiotic production. These has been some evidence for finger millets based probiotic production and their application. Therefore, to following objectives were defined.

1. Isolation of *Lactobacillus* from curd sample.
2. Characterization of *Lactobacillus.*
3. Production of finger millet beverage.
4. Determination of physico-chemical characters of beverage.
5. Determination of nutritive value of beverage.

CHAPTER-3

MATERIALS AND METHODS

❖ **Isolation of *Lactobacillus*:**

The *Lactobacillus* was isolated from curd sample. The curd sample was inoculated on sterile NRCL medium by four quadrant method. Followed by incubation in anaerobic jar for 2 - 3 days.

If isolation the organisms was confirmed based on morphological culture and biochemical characteristics.

❖ **Characterization of *Lactobacillus* :**

1. Colony characters

The colony characterization of the *Lactobacillus* bacteria on NRCL medium plate was observed and recorded.

2. Morphological Characteristics

The fresh suspension of isolate from the plate was used to study Gram staining by Hucker - Cohn (1923) Gram staining method and motility was observed by hanging drop motility test.

3. Biochemical characteristics:

Test for Biochemical characteristics of isolate was carried out as described in standard methods.

A. Catalase Test:

One ml of 30% of hydrogen peroxide was taken in a small test tube and growth of isolate was picked up with sterile nichrome wire loop and dipped into 30% hydrogen peroxide containing test tube for observations of no evolution of the gas bubbles i.e. test negative. (Rakesh Patel., 2008)

B. Methyl Red Test:

Loopful suspension of isolate was inoculated in to sterile glucose phosphate broth medium and were incubated at room temperature for 24 hours. After incubation 5 drops of the methyl red indicator was added. The test was negative indicated by development of no color in the medium.

C. Voges Proskauer test:

Loopful suspension of isolate was incubated into sterile glucose phosphate broth medium and was incubated at room temperature for 24 hours. After incubation 0.6ml of α-naphthol and 0.2ml of 40% of KOH were added. Test was negative indicated by development of no color in the medium.

❖ **Production of probiotic beverage**

The finger millets were thoroughly washed with distilled water and the impurities which appeared on the top of the water was discarded and kept for drying. Then roasted for 5 min. and fine powder of finger millet was made by using grinder. Then 25 gm powder of finger millets were cooked with 500ml water at temp 78^0 C for 30 min and cooled to normal temperature. After that the fresh culture of *Lactobacillus* (isolated from curd on NRCL medium) was inoculated in cooked millet and incubated at 37^0 C for 2 ,4 and 6 hrs. . Subsequently on the other hand 150 ml pasteurized milk, 55gm\liter Sugar and 5.1gm\liter coco powder were mixed together. This mixture was added to fermented finger millet solution, mixed well and filled in sterilized glass bottles and sealed it.

Sensory evolution was of the product conducted in order to select the best fermentation time. Different parameters like color, odor, taste, acidity, viscosity and overall acceptability was evaluated using 15 untrained persons. Finally, the highest scored beverage was selected for the proximate analysis and refrigerated. Physico-chemical (ph., titratable acidity, total reducing sugar, fat content) and microbiological parameters were evaluated.

❖ **Composition analysis:**

Protein content (Biuret method), reducing sugar content (DNSA method), ash content of milk

❖ **Physico – chemical analysis of the beverage:**

The pH. (pH meter), Viscosity (Viscometer)

❖ **Microbiological analysis of the beverage**

The total viable count of *Lactobacillus* bacteria was taken by spread plate count technique on MRS (De Man, Rogosa, sharpe's medium). The plates were incubated as at room temperature in an anaerobic condition using anaerobic jar for 2 to 3 days.

Catalase test, Gram staining test (Hucker - Cohn 1923), MR Test, VP Test, were performed for confirmation of *Lactobacillus*

❖ **Shelf life determination**

Sensory evolution for color, odor, acidity, flavor and over all acceptability were conducted once in two weeks. The pH and viscosity were tested per week for four consecutive weeks. The total viable *Lactobacillus* count was determined to evaluate the shelf life of probiotic finger millet beverage.

The total viable count of Lactobacillus was determined by serial dilution method. The beverage sample was serial diluted as 10-fold dilutions. 0.1 ml from each dilution was spread inoculated on MRS medium. All plates were incubated at room temperature in anaerobic jar for 2-3 days. After incubation colonies were counted on each plate.

CHAPTER NO. 4

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

❖ MORPHOLOGICAL CHARACTERISTICS:

The results of Gram staining and Motility are given in the following table.

- **Table no.1 Morphology**

Gram nature	Motility
Gram positive rod shaped arranged in chain	Non – motile organism

❖ CULTURAL CHARACTERISTIS:

The defining trait for identifying a bacterial species is typically the way the colony of bacteria looks on the culture media.

The observations of the colony characters are given in the following table.

- **Table no. 2 Colony Characteristics**

Size	Shape	Color	Margin	Surface	Elevation	Consistency	Opacity
1 mm	Circular	Dark pink	Entire	Smooth	Convex	Moist	Opaque

- **Photo No. 1: Culture Plate**

❖ BIOCHEMICAL TESTS:

From the observation table it can be concluded that the given organism shows positive results for MR test and negative results for Catalase, Caseinate and VP test

The results of biochemical tests are given in following table.

- **Table no.3 Results of biochemical tests**

Biochemical tests	Result
Catalase	-
Caseinate	-
MR	+
VP	-

'+' = Positive , '-' = Negative

❖ COMPOSITION ANALYSIS:

The composition analysis with respect to protein, reducing sugar and ash content of the beverage are given in following table

- **Table no.4: Composition Analysis**

Component	Percentage
Protein	1.73
Reducing sugar	7.55
Ash	0.65
Fat	0.09

- **Photo No. 2: Fermented Millet beverage filled in bottles**

❖ pH AND VISCOSITY OF PROBIOTIC BEVERAGE:

▪ pH of beverage:

The pH values of probiotic beverage in each week (4 weeks) are given the following table.

▪ Table no.5: pH of beverage:

Week	Observation
1	4.53
2	6.96
3	7.07
4	7.14

PH of beverage tested weekly, from observation it can be concluded that, as incubation period increases pH also increases.

❖ Graph:

- **Viscosity of beverage :**

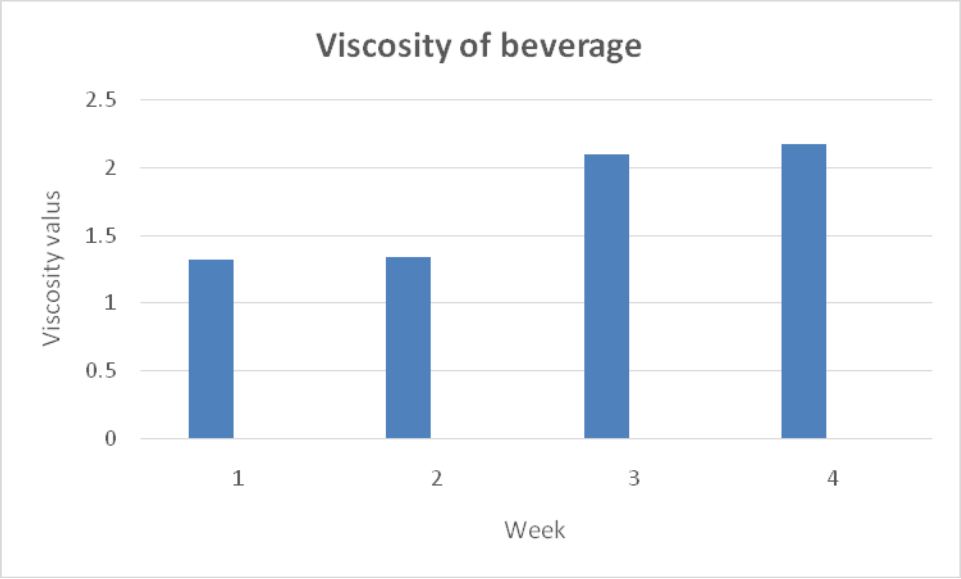
The viscosity values of probiotic beverage in each week (4 weeks) are given the following table.

- **Table No 6: Viscosity of beverage**

Week	Observation (Min.)
1	1.32
2	1.34
3	2.09
4	2.17

Viscosity of beverage tested weekly, from observation it can be concluded that, as incubation period increases viscosity also increases.

- **Graph :**



❖ **Protein concentration :**

The optical density of standard protein and sample are given in the following table.

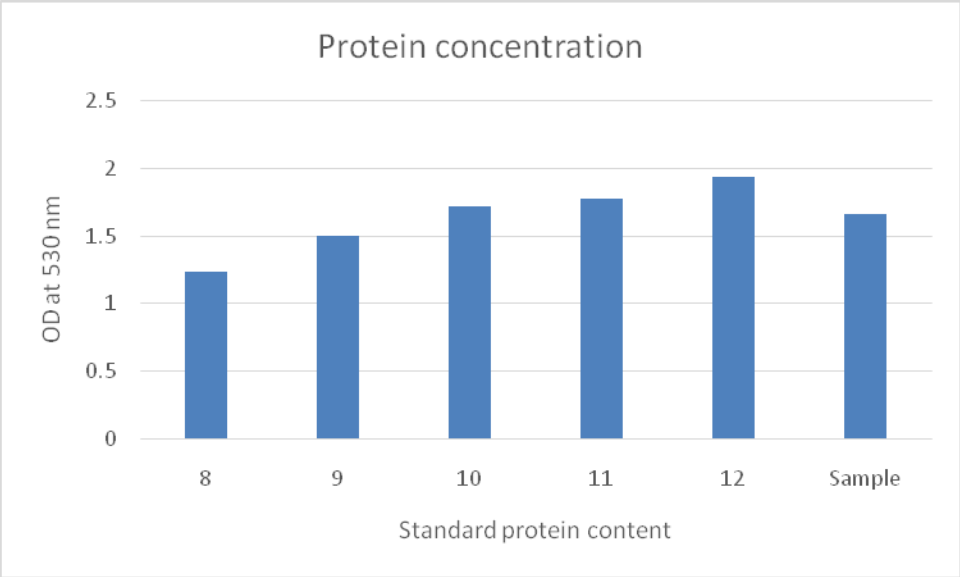
- **Table no. 7: Protein content of beverage**

Sr. No	Standard protein content	D/W	Biuret reagent	OD at 530 nm
1	8.0	5.0	3.0	1.24
2	9.0	4.0	3.0	1.50
3	10.0	3.0	3.0	1.72
4	11.0	2.0	3.0	1.77
5	12.0	1.0	3.0	1.93
6	**Sample**	5.0	3.0	**1.66**

The protein content of sample estimated by biuret method was from to be 1.73 %.

- **Photo No. 3: Biuret Test**

- **Graph :**



- **REDUCING SUGAR CONCENTRATION :**

The optical density of standard reducing sugar and sample are given in following table.

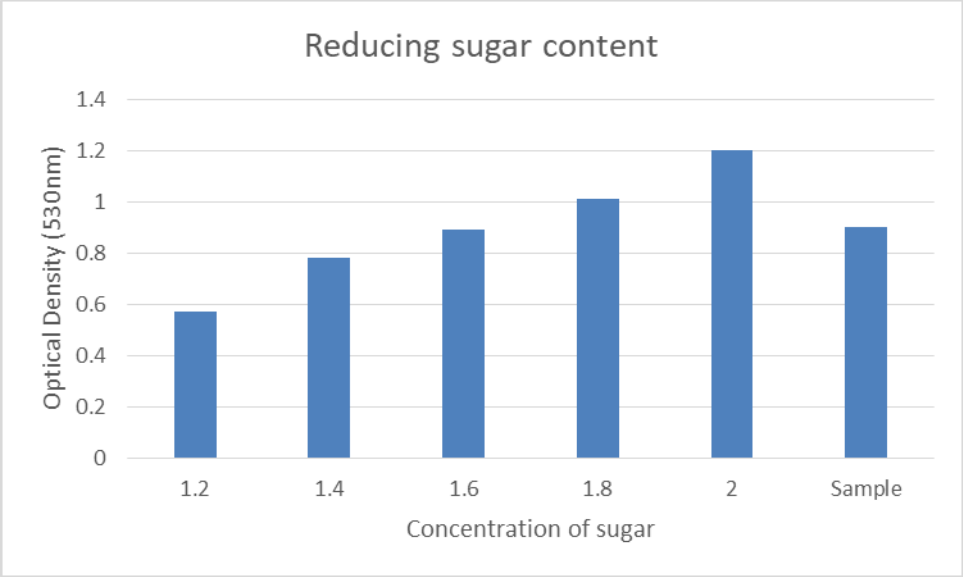
- **Table no.8: Reducing sugar content of beverage**

Standard glucose concentration	D/W	DNSA	OD at (530 nm)
1.2	0.8	2.5	0.57
1.4	0.6	2.5	0.78
1.6	0.4	2.5	0.89
1.8	0.2	2.5	1.01
2.0	00	2.5	1.20
Sample	0.8	2.5	0.90

The reducing sugar content of sample estimated by DNSA method was from to be 7.55%

- **Photo No. 4: DNSA Test**

- **Graph :**



CHAPTER- 5

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Probiotics are living microorganisms, that can have beneficial effects in human body. These microbes helps by supporting our body function and improve our health. In the present study, we focused on production of finger millet based probiotic beverage and determination of its nutritional value.

The *Lactobacillus* was isolated from curd sample on NRCL medium. This culture of *Lactobacillus* was further studied for its morphological ,cultural and biochemical characters.

The finger millets were thoroughly washed with distilled water and kept for drying. Followed by roasting for 3 minutes. Fine powder of roasted millet was done by using grinder and then this powder was cooked in water for 78 c for 30 minutes. After that fresh culture of *Lactobacillus* was inoculated in cooked millet and incubated at 37 c for 2, 4 and 6 hrs.Subsequently pasteurized milk, sugar and coco powder were mixed together. This mixture was added th fermented finger millet solution, mixed well and filled in sterile glass bottles and sealed it.

Sensory evaluation of product was done to select best fermentation time, The protein content,fat and reducing sugar content of beverage was determined. Similarly its pH and viscosity was also determined for 4 consecutive weeks, the total viable count of *Lactobacillus* was determined on MRS medium.

The satisfying results as per the pre-decided aims and objectives were obtained. However further application study of probiotic beverage in different conditions is necessary.

CHAPTER - 6

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CHAPTER- 7

APPENDIX

1. **NRCL Medium :-**

Composition :

Peptone	:	0.3gm
Meat extract	:	0.3gm
Yeast extract	:	o.3gm
Lactose	:	1gm
$Caco_3$	:	1.5 gm
1% Neutral red	:	0.5 gm
Distilled water	:	100ml
Agar-agar	:	2.5gm
pH	:	6.8

2. **MRS Medium (DeMan Rogosa and sharpe's medium):-**

Composition :

Peptone	:	1gm
Beef extract	:	1gm
Yeast extract	:	0.5gm
Glucose	:	2gm
Sodium acetate trihydrate	:	0.5gm
Polysorbate 80 \ Tween 80	:	0.1ml
Dipotassium hydrogen phosphate	:	0.2gm
Triammonium citrate	:	0.2gm
Magnesium sulfate heptahydrate	:	0.002gm
Manganese sulfate hydrate	:	0.005gm
Agar-agar	:	1gm
pH	:	6.2

3. **Milk agar :-**

Composition

tetraPeptone	:	1gm
Nacl	:	0.5gm
Meat extract	:	0.3gm
Agar-agar	:	2.5gm
pH	:	7.2
D\W	:	90ml
Milk	:	10ml

4. **Catalase test reagent :-**

10% hydrogen peroxide solution .

5. **Methyl Red Test:**

Composition:

Peptone	: 0.5
K_2HPO_4	: 0.5
D/W	:100 ml
Glucose 10% Soln	:5ml
pH	:7.6

6. **Grams iodine** (Lugols iodine) :-

Composition:

Iodine	:	1.0 gm
Potassium iodide	:	2.0 gm
Distilled water	:	100 ml

7. Crystal violet solution :-

Composition:

Solution A:

Crystal violet	:	20 gm
95 % ethanol	:	20 ml

Solution B:

Ammonium oxalate	:	0.8 gm
Distilled water	:	80 ml

8. Basic fuchsin Stain :-

Composition

Basic fuchsin	:	0.5 gm
Distilled water	:	1000 ml

9. DNSA

Composition

DNSA powder	:	1gm
NaoH	:	1gm
Phenol	:	0.2gm
Sodium sulphite	:	0.05gm
Potassium sodium tartarate	:	40gm
D\W	:	100ml