Volume II

"Microbiology: Recent Trends in Research Technology, Development and Future Aspects"

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“MICROBIOLOGY: RECENT TRENDS IN RESEARCH TECHNOLOGY, DEVELOPMENT AND FUTURE ASPECTS”

(Volume II)

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Book Title: **"Microbiology: Recent Trends in Research Technology, Development and Future Aspects"**

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PREFACE

In recent years, microbiology research has been rapidly advancing due to advancements in technology and the development of new techniques. The exploration of microbial communities associated with humans, animals, plants, and diverse ecosystems has provided profound insights into their role in health, disease, and environmental processes. These developments have opened up new avenues for studying microorganisms and have led to significant discoveries in various fields such as medicine, agriculture, and environmental science. This book aims to explores various aspects, including microbiome research, antimicrobial resistance, microbial ecology, microbial biotechnology, microbial genetics and genomics, microbial bioremediation, microbial nanotechnology, and microbial systems biology. By encompassing these diverse areas, the book offers a holistic view of the current state of microbiology research. Additionally, the book incorporates discussions on the ethical implications and challenges associated with microbiology research, adding depth and thought-provoking perspectives. Therefore, it will serve as a comprehensive guide for researchers, students, and enthusiasts who seek to understand the recent trends, advancements, and future directions in the ever-evolving field of microbiology. We hope that the insights provided within these pages will inspire new discoveries, foster collaboration, and ignite the imagination of those dedicated to unraveling the mysteries of the microbial world.

CONTENT

"Microbiology: Recent Trends in Research Technology, Development and Future Aspects" *https://www.kdpublications.in*

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1. Development and Quality Evaluation Dosa Prepared from Pearl Millet (*Pennisetum Glaucum*)

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Abstract:

Fermentation is the process of conversion of carbohydrates to alcohol and carbon dioxide using yeast, bacteria or combination of both under anaerobic condition. Dosa is the traditional fermented food which is prepared using cereal and pulse under anaerobic condition. Pennisetum glaucum is one of the top five millet in India. It is known as Pearl millet. In India it is commonly called as Bajra. The study was conducted to check how partial replacement of rice with pearl millet influence on the fermentation quality of dosa. Dosa prepared with 6 hours fermentation time had highest acceptability in terms sensory attributes and increased nutritive value. Proximate nutritional composition of the best accepted variation was analyzed by standard procedure. Dosa prepared from pearl millet

was rich in protein, fiber, calcium and phosphorus. Pearl millet being rich in dietary fibre with low glycaemic index reduces the risk of type-2 diabetes. Fermentation improves the digestibility and protect the gut health. Fermentation of millet is beneficial as it increases the availability of all essential ammino acids and antioxidants.

Keywords:

Fermentation, Pennisetum glaucum, low glycemic index, antioxidants.

1.1 Introduction:

Dosa is a rice-based crepe from fermented batter of rice and pulse which consumed as breakfast food and helps in easy digestion in the body. It is traditionally prepared in South-India which is usually served with chutney or sambar. Generally, it is a fermented product which is fermented with predominant microorganisms which are responsible for souring and gas production in the batter. Microorganisms involved fermentation of the batter are *Streptococcus, Pediococuscerevisiae, Leuconostomesenteroides* [1]. *Pennisetum glaucum* is known as Pearl millet. It is multipurpose cereal crop belongs Poaceae family. It is commonly called as Bajra, Bajri, Sajje, Kambu, etc in various Indian common languages. It is the third most important crop grown after rice and wheat in India. Commonly grown in Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Haryana [2]. Among all the millets, pearl millet has high content of macronutrients and significantly rich in starch and fiber, so it is notified as one of the millets under "Nutri-Cereal".

Hence it has very low glycaemic index reduces the risk of type-2 diabetes. Pearl millet is gluten-free; hence it is used as an alternative for the patients who have celiac disease [3]. Fermentation of pearl millet increases the nutritive value and improves the digestibility hence, protects the gut health. And provides all essential amino acids. Pearl millet has deep root system so it extracts soil nutrient and holds higher nutritional value than other millets. Hence, it is rich in iron, zinc, magnesium, copper, manganese, potassium and phosphorous. It is rich source of protein, dietary fibre and has high amount fat content compared to other cereal [4-5].

Figure 1.1: Pearl Millet (*Pennisetum Glaucum*)

1.2 Objectives:

- To develop Pearl millet dosa by partial replacement of rice with Pearl millet.
- To evaluate Organoleptic acceptability of developed product.
- To determine optimum fermentation time on the developed product.

1.3 Materials and Methods:

1.3.1 Raw Materials:

The present study was carried out in the Department of Food Science and Nutrition, Yuvaraja's College (Autonomous), University of Mysore, Mysuru. The raw materials such rice, black gram dhal, fenugreek seeds, pearl millet, salt were produced from the local market in Mysuru.

1.3.2 Method of Preparation:

Dosa was prepared by soaking different ratio of parboiled rice, black gram dhal, fenugreek seeds and pearl millet for 6 hrs. The soaked grains were ground into coarse paste and water was added to adjust according to the consistency of the batter. Then the batter was left for fermentation for 12hrs at room temperature. Heat the dosa pan and pour ladle full of batter and cook it on both sides by adding 2-3 drops of oil.

1.3.3 Optimization of Batter Fermentation:

The batter was allowed to ferment for different period (6, 12, 17 and 24 hrs) to check the fermentation quality in the batter. No effort was made to control the temperature during the process of fermentation.

1.3.4 PH and Volume:

For the different fermentation times and blend ratios of the Dosa batter properties viz. volume raised, pH was studied. The volume was recorded at 6, 12, 17 and 24 hrs with the help of measuring cylinder. The pH of the batter at a different fermentation time was recorded initially and at the end of fermentation using pH paper.

1.3.5 Nutritional Analysis of Prepared Peal Millet Dosa:

Standard AOAC (1980) method was used to determine the Nutritional composition of selected variation (P_3) of Pearl millet Dosa and control. The moisture content was estimated by using hot air oven at 98 to 100˚ C, protein content was estimated by determining total nitrogen content using standard Micro - Kjeld Hal method, ash % were estimated by high temperature incineration using muffle furnace and fat content was estimated by the Soxhlet method. The crude fibre content was estimated by crude fibre analyser. The carbohydrate content was obtained by subtracting from 100 with the sum of values of moisture, protein, fat and ash content per 100 g of the sample.

Minerals like Calcium, iron and phosphorous were analysed using inductively coupled plasma mass spectrometry (ICPMS). Then the pH of batter was determined using pH paper. These methods give a good precision and accuracy [6].

1.3.6 Statistical Analysis:

The sample was analysed in triplicates. The data obtained was analysed statistically using standard methods given by Snedecor and Cochran [7] and by Duncan's multiple range test with the $p \leq 0.05$ consider to be significant [8].

1.4 Sensory Analysis of Prepared Pearl Millet Dosa:

Sensory evaluation was carried out to determine the acceptability of various sensory attributes such as appearance, taste, colour, texture, taste, and overall acceptability. The product prepared was evaluated using 9-point headonic scale with 20 semi-trained panellists.

1.5 Statistical Analysis:

The sample was analysed in triplicates. The data obtained was analysed statistically using standard methods given by Snedecor and Cochran [7] and by Duncan's multiple range test with the $p \leq 0.05$ consider to be significant [8].

1.6 Flow Chart:

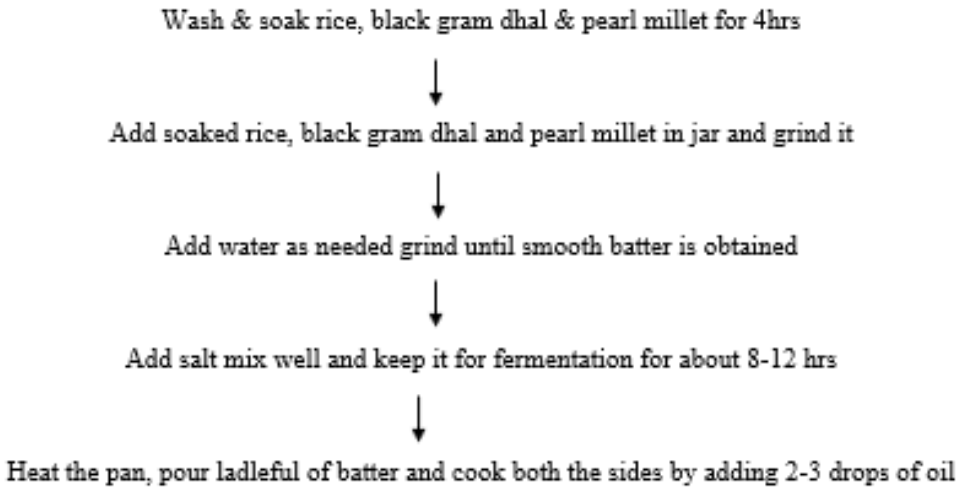

Figure 1.2: Flow chart for preparation of Pearl millet Dosa

1.7 Formulation:

Table 1.1: Standardization of Formula (ingredients g/100gm) for preparation of pearl millet dosa

Ingredients	P1 (standard)	P2 (20%)	P3 (40%)	P4 (60%)	P5 (80%)	P6 (100%)
Rice (g)	100	80	60	40	20	-
Pearl millet (g)	-	20	40	60	80	100
Black gram dhal(g)	25	25	25	25	25	25
Fenugreek seeds(g)	5	5	5	5	5	5
Salt (g)	3	3	3	3	3	3

Figure 1.3: Dosa prepared by varying the proportion of rice and pearl millet

Figure 1.4: Dosa Prepared by Varying the Fermentation Time

1.8 Results and Discussion:

1.8.1 Sensory Evaluation of Peal Millet Dosa:

The study was conducted to prepare millet based dosa by partially replacing rice with Pearl millet grains. The data pertaining to the effect of incorporation of various levels of Pearl millet (0%, 20%, 40%, 60%, 80%, 100%). The sensory attributes of dosa and results were shown in Table 1.2. The scores obtained for all sensory attributes P1, P2, P3, P4, P5 and P6. The variation P3 was more acceptable in terms of sensory attributes compared to other variations.

Table 1.2: Sensory evaluation of different variation of dosa developed from Pearl millet with partial replacement of rice. Values are mean ± SD, $p \leq 0.05$ (Holm Sidak method), n=20.

Variation	Appearance	Color	Texture	Taste	Flavour	Overall acceptability
P1 (control)	9±0.10	9±0	8.9±0.10	7.8±0.23	9±0.10	9±0.10
P2 (20%)	8.2±0.67*	7.9±0.25	7.9±0.25	7.8±0.23	7.8±0.23	7.9±0.25
P3 (40%)	8.7±0.20	8.5±0.45	8.03±0.65*	8.25±0.45	8.3±0.58	8.72±0.45
P4 (60%)	7.9±03	7.8±0.2	7.09±03	7.0±0.2*	7.25±015	7.22±0.25*

Variation	Appearance	Color	Texture	Taste	Flavour	Overall acceptability
P5 (80%)	7.5±0.1	7.4±0.2	7.25±0.33*	7.4±0.58	7.45±0.80	7.5±0.48
P6 (100%)	6.8±055	6.9±0.6	6.67±0.25	6.0±0.45	6.04±0.24*	6.5±0.61

1.8.2 Sensory Evaluation of Pearl Millet Dosa Prepared by Varying the Fermentation Time:

The accepted P3 variation was further incubated for different fermentation time to study the optimum fermentation time of Pearl millet incorporated dosa. The data pertaining to the effect of different fermentation time of Pearl millet grains (6 hrs, 12 hrs, 17 hrs, 24 hrs. The sensory attributes of dosa and the results were shown in the Table 1.3. The scores obtained for all sensory attributes of P1, P2, P3, P4, P5 and P6. The variation P3 was more acceptable in terms of sensory attributes.

Table 1.3: Sensory evaluation of prepared by varying fermentation time. Values are mean ± SD (n=30) *p value < 0.05 (Holm sidak method)

Parameters	6 Hours	12 Hours	17 Hours	24 Hours
Appearance	7.8±0.22	6.7±0.11	6.9±0.71	6.8±0.21
Color	7.5±0.40	6.4±0.75	6.7±0.51	6.7±0.11
Texture	6.9±0.11	6.6±0.12	7.2±051	6.7±0.90
Taste	7.0±0.05	6.5±0.33	6.6±0.55	6.4±0.80
Flavor	7.1±0.81	6.7±0.22	6.7±0.01	6.2±0.50
Overall acceptability	7.2±0.41	6.8±0.22	6.6±0.11	6.4±0.81

1.8.3 PH:

pH value of Dosa batter at different fermentation period ranged from 4.0 to 5.0 (6, 12, 17, 24 hours). Initial pH was 4.0, as fermentation occurred the pH was 5.0 throughout process even after varying the fermentation time.

There is an increasing in acidity level, i.e., decrease in the pH value. This is mainly due the development of Lactic acid bacteria producing lactic acid which lowers the pH, and production of carbon dioxide, which leavens the batter.

The accepted P3 variation was further incubated for different fermentation time to study the optimum fermentation time of Pearl millet incorporated dosa. The data pertaining to the effect of different fermentation time of Pearl millet grains (6 hrs, 12 hrs, 17 hrs, 24 hrs). The sensory attributes of dosa and the results were shown in the Table 1.4. The scores obtained for all sensory attributes of P1, P2, P3, P4, P5 and P6. The variation P3 was more acceptable in terms of sensory attributes.

Table 1.4: Determination of pH of Pearl millet dosa

Variation	Initial pH	pH after 6 hrs	pH after 12 hrs	pH after 18 hrs	pH after 24 hrs
P1 (control)	4	5	5	5	5
P1 20%	4	5	5	5	5
P2 40%	4	5	5	5	5
P3 60%	4	5	5	5	5
P4 80%	4	5	5	5	5
P5 100%	4	5	5	5	5

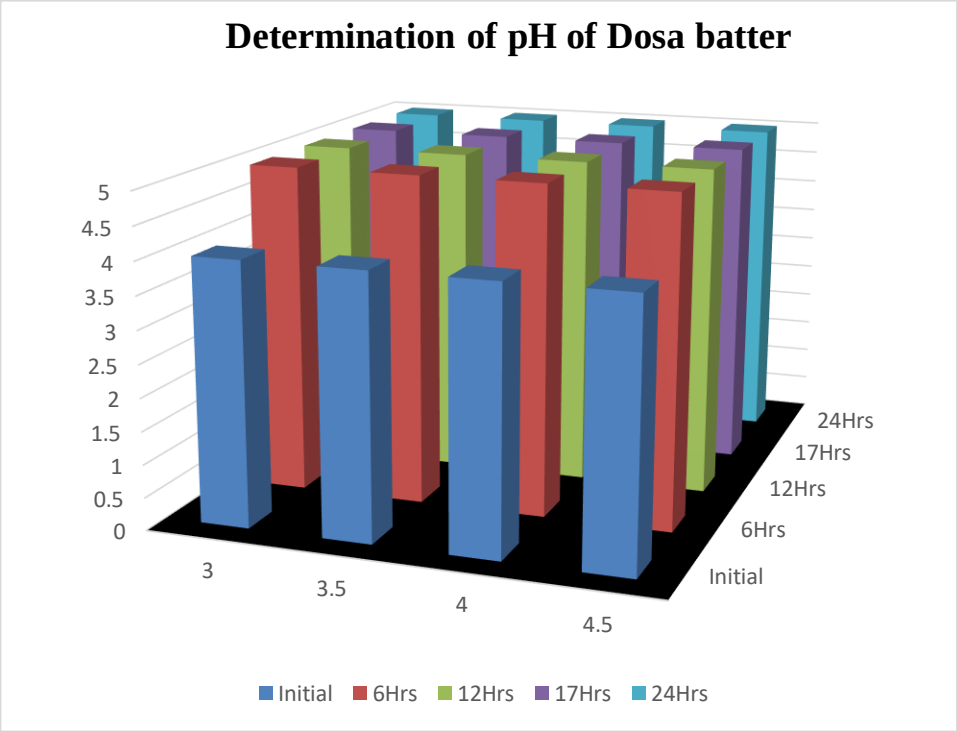

Figure 1.5: pH of Dosa batters

1.8.4 Optimization of Batter Fermentation:

The accepted P3 variation was further incubated for different fermentation time to study the optimum fermentation time of Pearl millet incorporated dosa. The data pertaining to the effect of different fermentation time of Pearl millet grains (6 hrs, 12 hrs, 17 hrs, 24 hrs). The sensory attributes of dosa and the results were shown in the Table 1.5. The scores obtained for all sensory attributes of P1, P2, P3, P4, P5 and P6. The variation P3 was more acceptable in terms of sensory attributes.

1.8.5 Proximate Analysis of Prepared Dosa:

The proximate composition of acceptable Pearl millet (P3) and that of control was analysed and the results were shown in the Table 1.5. The values of moisture, protein and dietary fibre content was increased compared to the control, whereas carbohydrate was less. However, ash, iron, and phosphorous content was also increased in Pearl millet dosa [6].

Table 1.5: Nutritional composition of selected variation (P3) of Dosa developed from Pearl millet with partial replacement of rice.

Values are mean ± SD, p ≤0.05 (Holm Sidak), n=3

Nutrients/100g	Standard Dosa	P3 (40%)
Moisture (%)	20.05±0.15	25.44±0.07
Carbohydrate (g)	57.7±1.108	50.4±0.19*
Protein (g)	18.82±0.03	21.1±0.09*
Fat (g)	1.04±0.25	1.36±0.02
Crude fiber (g)	0.74±0.01	0.95±0.25*
Ash (g)	1.65±0.37	0.4±0.04*
Energy (kcal)	315.44±0.27	270.02±0.27
Iron (mg)	3.69±0.02	5.31±0.64*
Phosphorus(mg)	257.75±0.05	274.51±0.02*

1.8.6 Volume:

Initial volume of the batter was 70 ml for all the variations and increased gradually till 18 hours. At 24 hours the volume of batter was reduced. This increased volume of Dosa batter is due to increase incorporation of the lactic acid bacteria into the batter during fermentation and entrapment of air.

Table 1.6: Determination of Volume of Dosa batter

Variation	Initial volume (ml)	Volume after 6hrs (ml)	Volume after 12 hrs (ml)	Volume after 18 hrs (ml)	Volume after 24 hrs (ml)
P1 (control)	70	80	85	87	70
P2 20%	70	80	84	80	78
P3 40%	70	80	85	78	76
P4 60%	70	74	87	71	77
P5 80%	70	72	72	74	71
P6 100%	70	70	70	70	70

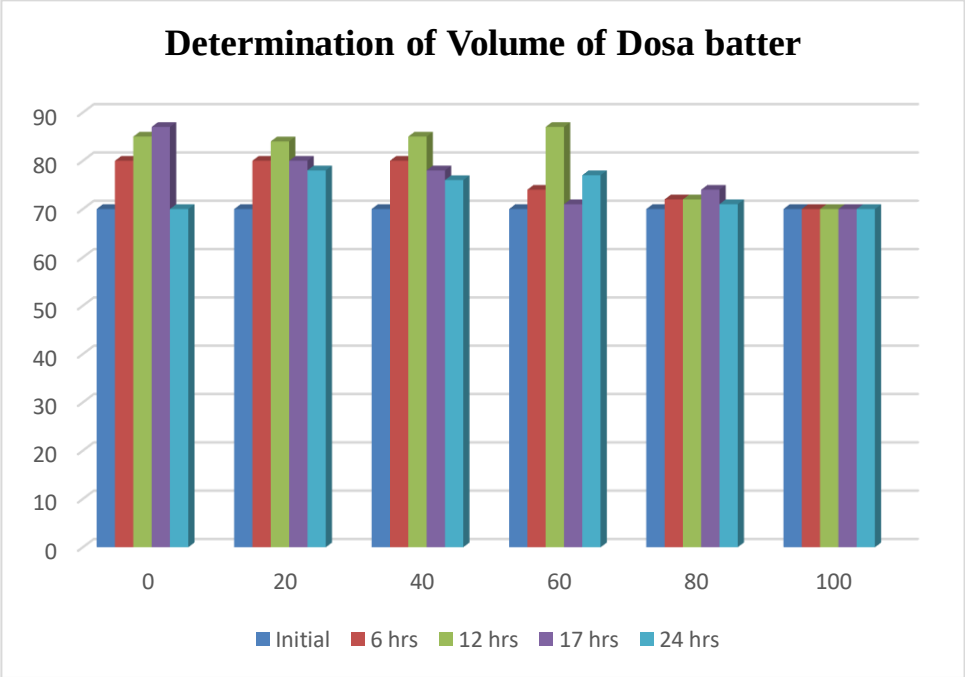

Figure 1.6: Volume of Dosa batters

1.9 Conclusion:

Pearl millet serves as a major staple food for many populations around the globe. Fermentation of millet improves the digestibility and increases the nutritional value of the millet. Millets naturally ferments from the wild yeast cultures. It increases the acidity and decreases the pH of the substrate thereby inhibits many pathogens. It enhances the sensory characteristics and improves the functionality qualities of food products and also reduces the cooking time of foods.

Microbes involved in fermentation are generally probiotic in nature and are good for enhancing the human gut health which includes *S. coccus, P. cerevisiae, L. mesenteroides.* It possesses probiotic and therapeutic properties and also increased the availability of all essential ammino acids and antioxidants.

Pearl millet of 40% (P3) had highest acceptability in terms of sensory scores compared to other variations. It had increased the levels of protein, dietary fibre, and phosphorous. The carbohydrate content was low compared to the control which was low in glycaemic index. The optimum fermentation time for Pearl millet dosa was 6 hrs and had up to 60% of acceptability.

1.10 References:

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2. Current Innovation in the Pharmaceutical Excipients Using Polyvinyl Pyrrolidone

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Abstract:

The N-vinyl-pyrrolidone on polymerization give a water-soluble polymer Polyvinylpyrrolidone (PVP). PVP is a polymer use as an excipient in formulation development of broad novel-controlled delivery systems. PVP use in formulation because it is an inert, non-toxic, temperature-resistant, pH-stable, biocompatible, biodegradable polymer that use to encapsulate and cater both hydrophilic and lipophilic drugs. Derivative of PVP has exceptional beneficial chemical properties these properties are depend upon the molecular weight. the PVP can conjugate with poorly soluble drug by Graft copolymerization or other techniques and release of drug can change to control or sustained release. the present chapter provide the mechanical, chemical, physiochemical properties and synthesis of derivative of PVP use in the drug, gene, and cosmetic delivery.

Keywords:

Polyvinylpyrrolidone, Povidone-iodine, Polymer, Drug delivery, Conventional dosage forms.

2.1 Introduction:

Polyvinyl pyrrolidone, also known as Povidone, is a linear polymer made up of the molecule N vinyl-pyrrolidone. PVP is nontoxic, non-ionic, inert, resistant to temperature changes, PH stable, biocompatible, and exhibits complex affinities for both hydrophilic and hydrophobic drugs.

These are the finest ideal characteristics. The Pharmacology of PVP As a substitute for shellac resin in hair spray, volume expanders are also used in medicinal cosmetics.

PVP plays a significant part in the biomedical, food, cosmetics, and pharmaceutical industries. PVP is interchangeable with 1-Ethenyl-2-Pyrrolidone, Povidone, Kollidon, Polyvidon, Polyvidonum, and Homopolymer. [1,2]

2.2 Physicochemical Properties [3,4]:

The following chart lists some of the physicochemical characteristics of PVP that make it appropriate for use in the pharmaceutical, biomedical, cosmetic, and food industries.

Table 2.1: Specific Properties of PVP

Sr. No	Property	Description
1	ICPUC	1-ethenylpyrrolidin-2-one
2	Molecular formula	$(C_6H_9NO)_n$
3	Molecular weight	2500–30,00,000 Da
4	Melting point	Softens at 150 °C and decomposes after 180 °C.
5	pH	3-7 (varies with K-value and concentration of solution)
6	Solubility	Soluble in water, ethanol, methanol, chloroform, acids, and amines. Insoluble in ethers, hydrocarbons, some esters, some ketones, and mineral oil.
7	Particle size distribution	Kollidon 25/30: 90% > 50 μm, 50% > 100 μm, 5%>200μm
8	CAS Number	9003-39-8
9	Chemistry	PVP polymer is comprised of functional groups C^{-} O, C–N, CH_2 with a strong hydrophilic moiety – pyrrolidone and a strong hydrophobic moiety – alkyl group
10	Water sorption	As the relative humidity increases, the water sorption and weight of PVP increases.

2.3 Preparation Methods of PVP and its Derivatives [5,6,7]:

Reppe's acetylene chemistry, developed at BASF, is the basis for PVP synthesis.

N-vinylpyrrolidone monomer is the primary raw material used to create PVP.

The synthesis is driven by a free radical polymerization process.

2.4 Synthesis of Monomer, N- Vinyl Pyrrolidone:

N-vinyl pyrrolidone is produced by the five-step Reppe process utilising acetylene as the starting ingredient.

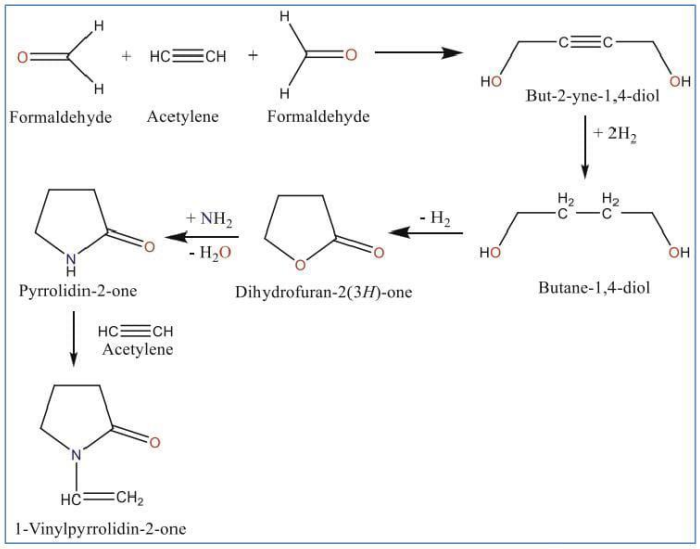

Figure 2.1: Synthesis of N-vinylpyrrolidone by following Reppe chemistry

2.5 Polymerization of Monomer:

PVP is obtained by processing the monomer N-vinylpyrrolidone by the Reppe method. PVP is made through a synthetic process and comes in three different types: homopolymers, copolymers, and cross-linked PVP.

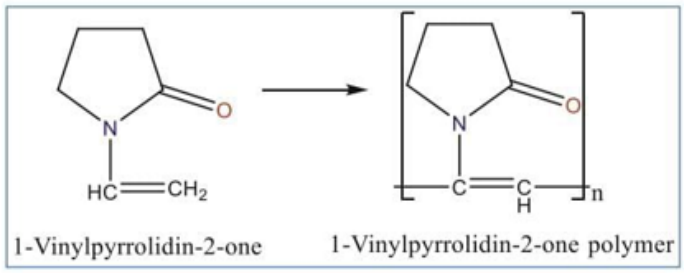

Figure 2.2: Reppe Method

2.6 Homopolymer Synthesis:

N-Free Vinylpyrrolidone Radical Mechanism This method uses soluble polyvinylpyrrolidine with a molecular weight of 2500 to 1 million by weight with an organic solvent or an aqueous medium. In this process, aqueous solutions such as water are used as an indicator to initiate the synthesis process of N-vinylpyrrolidone by free radical polymerization. At some final stage of polymerization, the process can produce different molecular weights of hydrogen peroxide by adjusting the concentration. The concentration of hydrogen peroxide affects the molecular weight of pvp, with a higher concentration of hydrogen peroxide leading to a decrease in molecular weight of pvp.

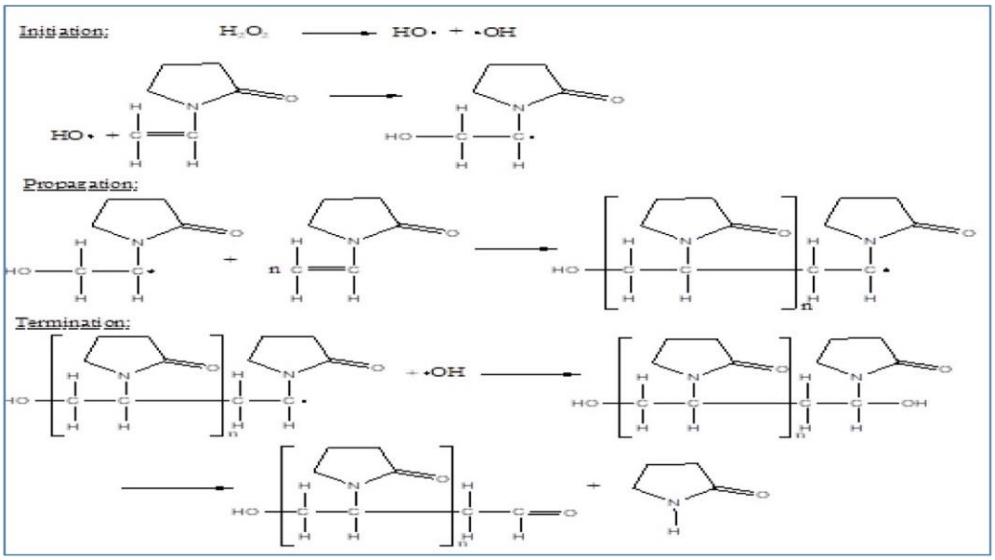

Figure 2.3: Homopolymer Synthesis

The synthesis of polymers is necessary to prepare an injectable low molecular weight soluble PVP. Polymerization is also possible with organic solvents such as alcohol, toluene, etc. The spray drying technique is used for the preparation of low and medium molecular weight pharmaceutical PVP, while drum drying/roll drying is used for the preparation of high molecular weight. process is used.

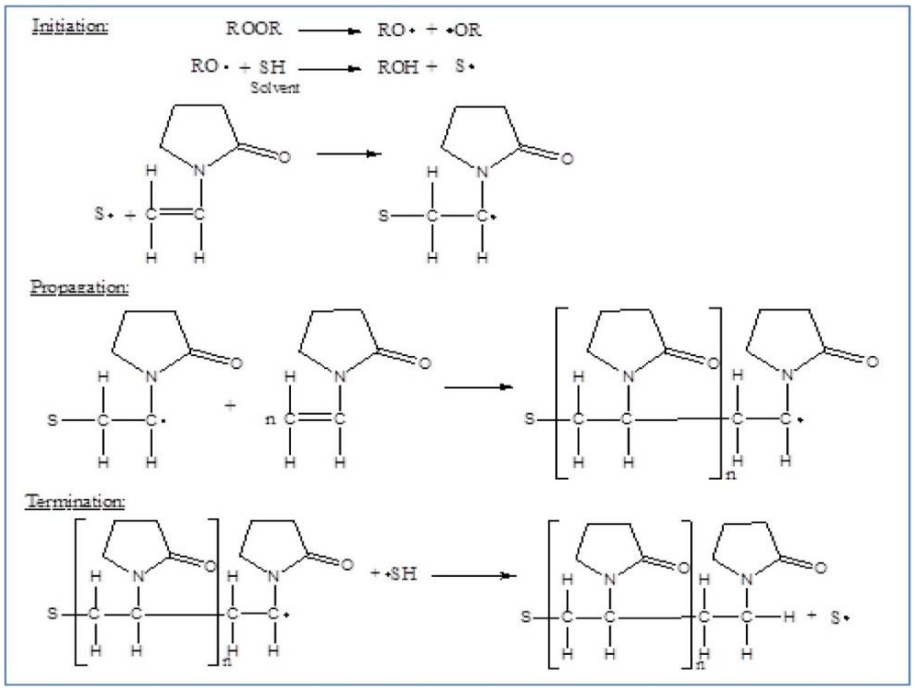

Figure 2.4: Polymerization with Organic Solvents

2.7 Copolymer Synthesis

The monomer is used in the copolymerization process of its copovidone synthesis [for example, vinyl pyrrolidone-vinyl acetate copolymer]. Anionic and cationic copolymers are two different types. Anionic copolymer can be used as a release agent because it dissolves in oil, while cationic copolymers are used in cosmetics, such as hair rollers, because cationic polymers have better adhesion properties.

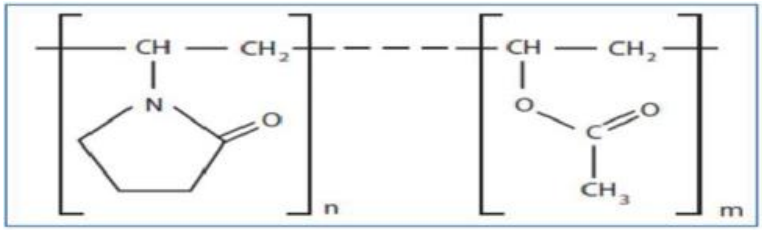

Figure 2.5: Copolymer Synthesis

2.7.1 Cross-linked Polymer Synthesis:

In the synthesis of cross-linked polymer, any other amount of N-vinylpyrrolidone undergoes popcorn polymerization by two methods, first using alkaline hydroxide at > 100 ºC or adding the dysfunctional monomer directly to water at 100 ºC and insoluble cross-linked.

2.8 Characterization Methods of PVP [8,9]:

The method of characterization and analytical determination of PVP is clearly indicated in the literature and monographs of many pharmacopoeias. An important analytical method for the detection of PVP is infrared spectroscopy. Other test can be used to determine the exact type of PVP, such as soluble morphology studies, molecular weight, particle size and viscosity. The pharmacopoeia lists many chemical tests for the quantitative measurement of PVP as well as for the identification and qualitative evaluation of PVP. Several techniques have been described in the literature, including photometry, decisive redness, turbidity, and gas chromatography. Hydroxyl acid detection and electrophoresis, paper chromatography, infrared spectroscopy, gravimetric analysis, complication capacity and solubility, high performance liq.

2.9 Pharmacokinetics, Toxicology and Safety:

Working out the pharmacokinetics and toxicity of PVP required a lot of research. After the introduction of the kinetic profile of PVP, it was studied in various animal models and in humans, focusing on the pharmaceutical, pharmaceutical and food industries. Pharmacokinetics

According to various studies, the molecular weight of the polymer and the transport route affect the absorption, distribution, metabolism and excretion of PVP. According to studies, absorption of PVP in ferrets, humans and rabbits is either non-existent or quite low. PVP is

absorbed in the reticuloendothelial system. According to studies, the metabolism of PVP has not been proven. Blood and urine samples taken after intravenous administration of PVP to rats, dogs, and humans showed no evidence of metabolism. Low molecular weight PVP is excreted by the kidneys after intravenous injection. Toxicology

There are so many reports claiming that PVP is biologically inert, non-toxic and well tolerated those toxicological studies in animals such as rats, pigs, dogs and rabbits administered orally to PVP in some cases found no acute, sub-chronic or. chronic toxicity. . However, PVP has been shown to cause diarrhoea in some cases at high doses. Due to the high content of PVP, which has an osmotic effect and acts as a laxative, diarrhoea is induced. Histopathology, haematology and clinical chemistry show no toxicity. When administered intravenously or intraperitoneally, high molecular weight PVP is retained in RES tissue. The reason for the retention can be related to the inability of the glomerulus to filter large molecules. PVP LD50 for oral ingestion.

2.9.1 Security:

According to toxicological analysis, PVP is a physiologically inert material that is safe for use in food, medicine, and pharmaceutical applications in both humans and animals. The recommended daily amount of PVP is 50 mg/kg/day. PVP can be used topically and orally without side effects. In terms of molecular weight and route of administration, copovidone and crospovidone were also found to be safe, and no toxicity was observed with parenteral administration of PVP, so repeated administration is recommended.

2.9.2 Pharmaceutical and other Applications [10,11,12]:

PVP as an excipient in the development of for Conventional dosage forms. Several Conventional dosages forms.

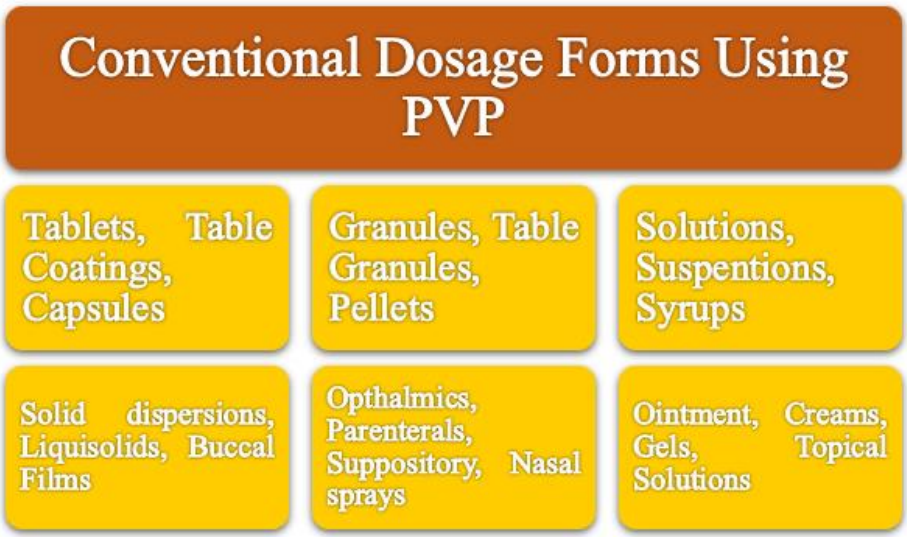

Figure 2.6: Conventional dosage forms using PVP

2.10 Oral Solids:

Tablets, Tablet Coating, Capsules, Granules, Pellets, Solid Dispersions, Ducal Films, and Many Traditional Dosage Forms are examples of oral solids.

The amorphous drug's improvement of poorly soluble drug solubility Noncomplex, a poly electrolyte, is made by Dong et al. The crystallisation tendency of some drugs causes the existing nanoparticles to become unstable after prolonged storage; therefore, PVP is used as a crystallisation inhibitor in the drug electrolyte noncomplex to create hollow gold Nano shells [HNG] off roadenium using biodegradable polymer poly lactic acid and PVP as a stabiliser.

Gudrun et al. created the paclitaxel magnitude electric nanoparticle using PVP as a component. Jived et al. have demonstrated that the PVP employed to provide field control has good selectivity for medication delivery to ovarian cancer cells.

2.10.1 Tablet Coating:

They have small cracks while sugar coating. Micro-PVP is therefore used due to its film-forming nature and is also hydrophilic and applied for better adhesion of the coating over the hydrophobic core.

In the presence of PVP, the colour solutions or suspension has a uniform distribution, and its dispersion effect in the coating mixture representing PVP also has the property of preventing the growth of crystals to maintain the stability of the sugar suspension.

PVP is also used in tablet film coatings. In the film former, PVP is not used alone as a film former in coating mixtures because of its hygroscopicity. For tablet enteric coating, PVP is also used as an additive with the enteric coating polymer

2.10.2 Granules/Granules/Capsules:

Granular, granulated, effervescent, chewable dry syrup in this Pp. is used as a binder and also when filling tablets or hard gelatine capsules or as a coating. Using a roller with dry granulation technology. Sealant PVP KEO is used as a binder in the production of acerbate granules. Soft Gelatine Capsules in this highly concentrated solution can be filled and prepared with PVP.

2.10.3 Solid Dispersion:

In various formulations, PVP has many functions, such as being used as a solubilizing agent to improve solubility and increase the bioavailability and dissolution rate of poorly soluble drugs. PVP plays an important role in adding amorphous drugs and thus in a solid state, which prevents crystallization and. Retention of super saturation of drugs after dissolution. PVP is hydrophilic in nature and forms water-soluble complexes with the low molecular weight drug, which also prevents crystallization in the dissolved drug.

2.10.4 Liquisolid Compacts:

In Liquisolid technology, PVP was used as an additive to improve the dissolution rate of poorly soluble drugs. To improve the dissolution rate of the poorly water-soluble drug ketoconazole by preparing a liquid-solid concentration, this study was conducted by Morale et al. Here he uses various excipients, such as microcrystalline cellulose as a carrier and colloidal silicon dioxide as a coating material. PVP as an additive and PEG 400 as a non-volatile solvent.

Another work showed an increased dissolution rate of carbamazepine due to PVP as an additive in liquid - Solid Compact and that Compact Made, which is made by direct compression technique with the simplest technique. He claims that PVP prevents precipitation of the drug from the supersaturated substance. liquid drug, preventing crystal growth and also providing a large surface area against the dissolution medium due to adsorption to the carrier.

2.11 Oral Membranes:

Buckle films: Fast-dissolving buckle films using petroleum-based HPMC and PVP K30 were developed and evaluated by Allogamy et al. For different types, the films can be loaded first with the drug in its pure form, or secondly with the co-ground form using citric or tartaric acid, and finally with a self-micro emulsification system, where labra sol and Tween 80 mixed with polio pique have developed a new crystalline structure for the film developer aftopidil.

Used HPMC PVP. The new crystalline nature of the drug must be discarded in the presence of organic acids or emulsifying systems. A rabbit model is selected for in vitro or in vivo evaluation. This model has great importance in improving the dissolution rate and bioavailability in the selected rabbit model, and the drug is administered as an oral film.

2.12 Oral Liquids:

Oral liquids consist of various preparations, such as oral drops, solutions, suspensions, dispersions, emulsions, and PVP polymer is used in oral liquids in cardboard boxes, for example, it acts as a solubilize for several poorly soluble drugs, such as diclofenac.

For an oral liquid dosage form, the desired viscosity can be achieved and may be required to achieve a constant drop, dispersion, improved appearance and physical stability.

The required medium as well as high molecular weight PVP acts as a thickener containing acetaminophen, trimethoprim and sulfamethoxazole, in this PVP is used to achieve a taste masking effect.

In oral liquid, there is a possibility of crystal growth in cap-lock syrups, therefore PVP is used as a crystal growth inhibitor in oral liquid to prevent crystallization in various oral liquid dosage forms.

2.13 Suspensions / Emulsions / Dispersions:

In many oral liquid dosage forms such as dry syrups, suspensions, emulsions, dispersions, PVP has a wide range of applications such as suspending, dispersing, stabilizing and increasing viscosity. PVP effectively stabilizes suspensions, emulsions acting as protective colloids and dispersions by adsorbing as a thin molecular layer on the surface of individual colloidal particles and thus prevents their aggregation or coalescence and also avoids contact with particles.

The individual solid particles are held apart from each other by steric hindrance and therefore also become hydrophilic. According to Stake's law, PVP increases the viscosity, so the settling velocity decreases, so the viscosity is inversely proportional to the settling velocity. If the sediment volume is increased, better spread ability is achieved. Zeta potential of substances such as iron oxide pigment can be reduced by adding PVP and thus improves suspension stability for dissolution Suspension or preparation of dry syrups and also soluble granules. PVP is used as a binder. Suspension for parental use. Dosage must be endotoxin-free, so low molecular weight endotoxin-free grade PVP is used in oral suspension formulations, while medium and high molecular weight PVP is used.

2.14 Ophthalmological:

The contact time of the eye product can be extended by using PVP and thus PVP is also used to increase the viscosity of PVP in d-drug solution and also as an effective lubricant for dry eyes. PVP was also used to prevent crystallization where PVP acts as a crystal growth inhibitor. PVP is widely used in ophthalmology to obtain clear solutions. ophthalmic PVP acts as a solubilizing agent and also improves the effect and bioavailability of the active ingredients.

PVP reduces the irritant effect of oxymetazoline, so PVP was also used to reduce eye irritation. PVP is used to prepare a solution for cleaning contact lenses. In ophthalmic suspension, PVP acts as a stabilizing, suspending or thickening agent. High molecular weight classes are mostly used in contact lens solution and low molecular weight classes are used as eye drops, some drugs used in eye preparations with PVP are for example chloramphenicol, pilocarpine, prednisolone, etc.

2.15 Parenteral:

In human or veterinary parenteral products, PVP polymer is used to dissolve or disperse drugs in injectable products. Parenteral products must be free of endotoxins. Thus, low molecular weight endotoxin-free or pyrogenic-free PVP is easily removed after parenteral administration.

High molecular weight PVP and other types of PVP such as K12 or K17 are also used. PVP is used as a solubilize when injecting antibiotics, rifampicin prepares injections for better solubility and stability in the lyophilisation process. PVP is used a lot. With intracytoplasmic injection, the viscosity of PVP is improved.

2.16 Novel Drug Delivery Systems Using PVP [13,14,15]:

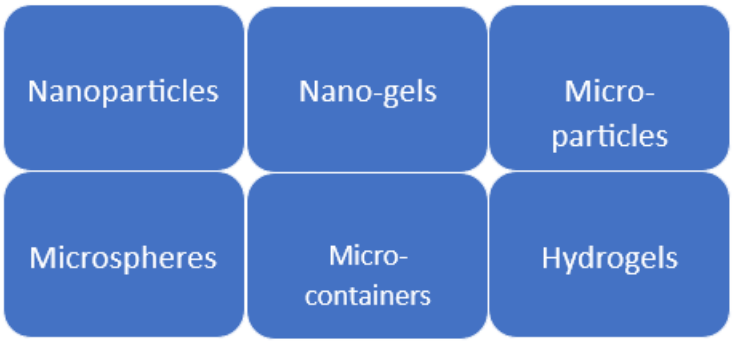

Figure 2.7: Novel Drug Delivery Systems Using PVP

2.16.1 Nanoparticles:

the amorphous drug's improvement of poorly soluble drug solubility Noncomplex, a poly electrolyte, is made by Dong et al. The crystallisation tendency of some drugs causes the existing nanoparticles to become unstable after prolonged storage; therefore, PVP is used as a crystallisation inhibitor in the drug electrolyte noncomplex to create hollow gold Nano shells [HNG] off roadenium using biodegradable polymer poly lactic acid and PVP as a stabiliser.

The magnitude of the electric nanoparticle of paclitaxel using PVP as a component is prepared by Gudrun et al. Using PVP, high specificity drug delivery to ovarian cancer cells was made possible.

2.16.2 Nano Gels:

The angels are formed by Gamma radiation induced polymerization with acrylic acid in aqueous PVP solution as a polymer the Nano gels are formed by cross link aging complication mechanism and hydrogen bonding interaction the molecular weight of PVP is an important parameter which influence the size and swelling ability of Nano gels.

By using PVP and an aqueous pool of hexadecyltrimethylammonium bromide reverse micelles, Buenos et al. developed a superabsorbent angel with a tenfold swelling ratio.

The swelling was dependent on bound Fe3+ and varied with pH and ionic strength.

2.16.3 Microspheres/Microcapsules:

Prepare a drug's ternary complication system using PVP. The factorization during the development of chitosan-calcium alginate microspheres is improved by the ternary complex system prepared with celecoxib, hydroxypropyl cyclodextrin, and TV pike 32.

The results demonstrate that the ternary complication system has a higher drug dissolution rate than the binary system, which in turn has a higher dissolution rate than the pure drug alone.

2.16.4 Micro Containers:

The idea of impregnation with supercritical fluid and inkjet printing combined. PVP is used as a polymer, and ketoprofen is a model medication. As the medicine is impregnated in polymer matrix supercritical fluid carbon dioxide as the loading medium, PVP solution is spread into micro container with a quasi-no-waste process using inkjet printing. The repeatability of such drug-loaded polymer micro containers was shown to have a higher dissolution rate than solid dispersion made with the same substance.

2.16.5 Hydrogels:

PVP is used to develop a hydrogel because it swells in an aqueous environment. A polymeric hydrogel prepared by Oliveira et al. using those containing PVP K90. Chitosan and clay as nanoparticles using gleans as a model drug. Chitosan-drug interaction b Studied and reported. Drug release is affected by the swelling rate of the PVP clay system and the chitosan-PVP clay system. Cross-linked PVP hydrogels with three different compositions were prepared by Ahmad et al. As a result, the increased concentration of PVP decreased the drug release from the hydrogel-based compacted matrix tablet, using PVP-polyacrylic acid copolymer, Jin et al developed a new pH and electrical sensitive hydrogel. This pH- and electro-sensitive hydrogel is suitable for use in actuators, switches and drug delivery systems.

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3. Development and Quality Evaluation of Dhokla from Pearl Millet (*Pennisetum Glaucum*)

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Abstract:

Fermentation is any metabolic process in which microorganisms' activity creates a desirable change in food products. Dhokla is a fermented food of India and is popular throughout the country. Pearl millet is an energy packed grain. It is rich in essential nutrients such as protein, dietary fiber, phosphorus, magnesium and iron. this study was conducted to develop and evaluate fermented dhokla of six different compositions P1, P2, P3, P4, P5, P6 containing Bengal gram dhal, curd with different proportions of pearl millet (0%,20% ,40%,60%,80%,100%) were developed and evaluated for acceptability using subjective and objective method by taking Bengal gram dhal dhokla as a control. these developed products were analysed for sensory attributes (n=20) and the sensory score is

highest for P3 and its nutrient composition was recorded. Then fermentation time was varied in P3 formulation for 6, 12, 18, and 24 hours for evaluation of fermentation quality. The sensory score was highest for the product which was fermented for 6 hours. Dhokla prepared from Pearl millet had more protein, fibre and iron. They are gluten free and have low glycaemic index compared to traditional Dhokla, since they contain less carbohydrates and more fibre.

Keywords:

Pearl millet, dietary fiber, fermentation, glycaemic index,

3.1 Introduction:

Dhokla is a traditional legume-cereal based fermented food, it is a Gujarati dish which is widely popular due to its texture. It is prepared by soaking Bengal gram dal and rice separately. The soaked ingredients are ground in to fine paste, common salt and curd is added as inoculum, and they are mixed thoroughly. The batter is kept for overnight fermentation.

Leavening of batter is due to the development of acid flavour, indicating fermentation. Microbiota that are associated with the fermentation are *Leuconostoc mesenteroides, Lactobacillus fermentum, Pichia silvicola, Candida sp., Streptococcus faecalis, Torulopsis candida* and *Torulopsis pullulans.*

The lactic acid bacteria (LAB) are the important bacteria that are involved in the development of characteristic flavour of dhokla, while the yeasts are also involved in the raising the batter volume by producing folic acid, hence they give the spongy texture to the dhokla when they are cooked in steam (1).

Pearl millet is the sixth most important cereal crop after rice, wheat, maize, barley and sorghum (2). It is also considered one of the crops that can provide good nutrition and income to small-scale farmers (3). It has a deep root system and can survive in a wide range of ecological conditions under water scarcity. It has high photosynthetic efficiency with an excellent productivity and growth in low nutrient soil conditions and is less reliant on chemical fertilizers (4). Mineral-wise, it is a rich source of iron, zinc, magnesium, copper, manganese, potassium and phosphorous. Mature kernels are rich in vitamin A but deficit in vitamins B, and C (5). The niacin content is significantly high even after dehulling; hence millet consumers do not suffer from pellagra, the niacin insufficiency disease (6). In comparison to maize or wheat that are uncultivatable in harsh conditions, pearl millet is cultivatable in areas with drought, low soil fertility, high salinity, low pH or high temperature. Even in case of climate change with harsh temperature conditions, pearl millet is adaptable (7). It is almost free from major diseases and insect attack; it could be cultivated with good harvest (8). The food value of pearl millet is superior to other cereals in its protein content with an excellent balance of amino acids and relatively high vitamin A content (9, 10). It helps to keep blood sugar levels stable for a long time in diabetic patients. It is also helpful for diabetes patients because it has a comparatively small glycaemic index that helps steadily digest and contain glucose at a slower pace than other foods (11).

3.2 Objectives:

- To develop Pearl millet based Dhokla by partially replacing Bengal gram dhal with Pearl millet.
- To evaluate the organoleptic acceptability of the developed product.
- To determine optimum fermentation time on the developed product.

3.3 Materials and Methods:

3.3.1 Raw Materials:

The present study was carried out in the department of food science and nutrition, Yuvaraja's college, (autonomous) University of Mysore, Mysore. The raw materials were procured from local market of Mysuru such as Pearl millet, Bengal gram dhal, curd, salt, oil, turmeric powder, chillies, mustard seeds and curry leaves.

3.3.2 Method of Preparation:

The standardisation of Dhokla was done by varying the proportion of Pearl millet and Bengal gram dhal. The cleaned Pearl millet and Bengal gram dhal was soaked for 6 hrs in water followed by discarding the soaked water and grounding them into fine paste and water was added to get require consistency. Salt (NaCl) and curd was added and were allowed to ferment for 12 hrs in anaerobic condition at room temperature. To the fermented batter pinch of turmeric powder, 1tsp of green chilli paste and a pinch of soda was added followed by steam cooking for 30 min, then they were cut into square pieces. They were seasoned with oil, mustard seeds, asafoetida, curry leaves and green chillies, Pour this mixture on the dhokla.

3.3.3 Optimization of Batter Fermentation

After addition of salt 2% of total weight of raw material, the batter was allowed to ferment for different period (6, 12, 18, 24 h) in a stainless-steel vessel.

No effort was made to control the temperature during the process of fermentation.

3.3.4 Ph and Volume

For the different fermentation times and blend ratios of the Dhokla batter properties viz. volume raised, pH was studied. The volume was recorded at 6, 12, 18 and 24 hrs. with the help of measuring cylinder. The pH of the batter at different fermentation time was recorded initially and at the end of fermentation using pH paper.

3.3.5 Sensory Analysis of Dhokla:

The developed dhokla was evaluated for its organoleptic properties like appearance, colour, texture, taste, flavour and overall acceptability. 30 semi-trained panellists were involved in

sensory evaluation and 1-to-9-point hedonic scale was used for rating the quality of the dhokla. The mean value of 30 scores was considered for evaluation.

3.3.6 Nutritional Analysis of Prepared Dhokla:

The proximate estimation was carried out by following standard AOAC (1990) method for chosen variation P3 (40%) & control. These methods have good accuracy & precision.

The moisture content was evaluated using hot air oven at 98-100 degree Celsius, whereas protein content was estimated using standard Micro-Kjeldhal method by determining total nitrogen content, fat was analysed by using Soxhlet method and ash (%) was estimated by incinerating the food sample at high temperature (550 degree Celsius for 6hrs) in muffle furnace. The crude fibre was estimated by Crude Fibre Analyser. The carbohydrate content was evaluated by subtracting from 100 with the sum of the values of moisture, protein, fat, and ash content per 100 g of the sample (12).

Calcium and iron were estimated by inductively Coupled Plasma Mass Spectrometry (ICPMS) (9).

3.3.7 Statistical Analysis:

Each sample was analysed in triplicates. The data obtained was analysed statistically using standard methods given by Snedecor and Cochran (13) and by Duncan's multiple range test with the $p \leq 0.05$ considered to be significant (14, 15).

3.4 Formulation of the Product:

Table 3.1: formulation of the products (ingredients g/100 g) for preparation of Pearl millet dhokla

Ingredients	Standard	20%	40%	60%	80%	100%
Pearl millet (g)	-	20	40	60	80	100
Bengal gram dhal (g)	100	80	60	40	20	-
Salt (g)	2	2	2	2	2	2
Curd (g)	15	15	15	15	15	15
Turmeric (g)	1	1	1	1	1	1
Mustard seeds (g)	2	2	2	2	2	2
Curry leaves (g)	5	5	5	5	5	5
Chillies (g)	2	2	2	2	2	2

Cleaned Pearl millet

↓

Soak Bengal gram dhal and millet for 4 h

↓

Drain and grind in mixer grinder to get coarse batter consistency

↓

Add salt (2%) and curd to the batter

↓

Natural fermentation for 12 h at room temperature

↓

Add turmeric and green chilli paste to the batter

↓

Pour the batter into plates

↓

Steam cook for 15 min

Figure 3.1: Flow Chart for Preparation of Pearl Millet Dhokla

Figure 3.2: Different variations of Dhokla developed by partially replacing bengal Gram Dhal with Pearl Millet.

3.5 Result and Discussion:

3.5.1 Sensory Evaluation of Pearl Millet Dhokla:

The study was undertaken to prepare millet based Dhokla by partially replacing Bengal gram dhal with Pearl millet. The data pertaining to the effect of incorporation of various

levels of PMD (20, 40, 60, 80 and 100%) on sensory attributes of Dhokla and the results are shown in Table 3.2. The scores obtained for all sensory attributes for P2, P3, P4, were similar to the control, whereas P5 and P6 showed decreased score and were less acceptable compared to the other variations. The acceptable P3 variation was incubated for different fermentation time to study the optimum fermentation time of Pearl millet incorporated Dhokla.

Table 3.2: Sensory evaluation of different variation of Dhokla developed from Pearl millet with partial replacement of Bengal gram dhal.

Variation	Appearance	Colour	Texture	Taste	Flavour	Overall acceptability
P1 (control)	9.12 ± 0.41*	9.23 ± 0.14	9.13 ± 0.16	8.92± 0.12	8.94± 0.16	9.21 ± 0.15
P2 (20%)	8.12 ± 0.32	7.31 ± 0.23	7.15 ± 0.12	7.12± 0.26	7.69± 0.23	7.23 ± 0.12*
P3 (40%)	8.45 ± 0.32	8.21 ± 0.23	7.92 ± 0.12	8.12± 0.26	8.23± 0.23	8.46 ± 0.12*
P4 (60%)	7.91 ± 0.24	7.82 ± 0.33	7.92± 0.41*	7.87± 0.41	7.71± 0.26	7.81 ± 0.63
P5 (80%)	7.41 ± 0.14	7.52 ± 0.14	7.13 ± 0.36	7.21± 0.14	7.02±0.13*	7.22± 0.36
P6 (100%)	7.31 ± 0.22	7.21 ± 0.31	7.22 ± 0.42	7.01± 0.36	7.21±0.12	7.34± 0.13*

Values are mean SD, p ≤0.05 (Holm Sidak method), n=20

3.5.2 pH

Table 3.3: pH of Dhokla batters

Variation	Initial pH	pH after 6 hrs	pH after 12 hrs	pH after 18 hrs	pH after 24 hrs
P1 (Standard)	6	5	5	5	5
P1 20%	6	5	5	5	5
P2 40%	6	5	5	5	5
P3 60%	6	5	5	5	5
P4 80%	6	5	5	5	5
P5 100%	6	5	5	5	5

pH value of Dhokla batter at different fermentation period ranged from 5.0 to 6.0 (6, 12, 18, 24 hours). Initial pH was 6.0, as fermentation occurred the pH was 5.0 throughout process even after varying the fermentation time. There is an increasing in acidity level, i.e., decrease in the pH value. This is mainly due the development of Lactic acid bacteria producing lactic acid which lowers the pH, and production of carbon dioxide, which leavens the batter.

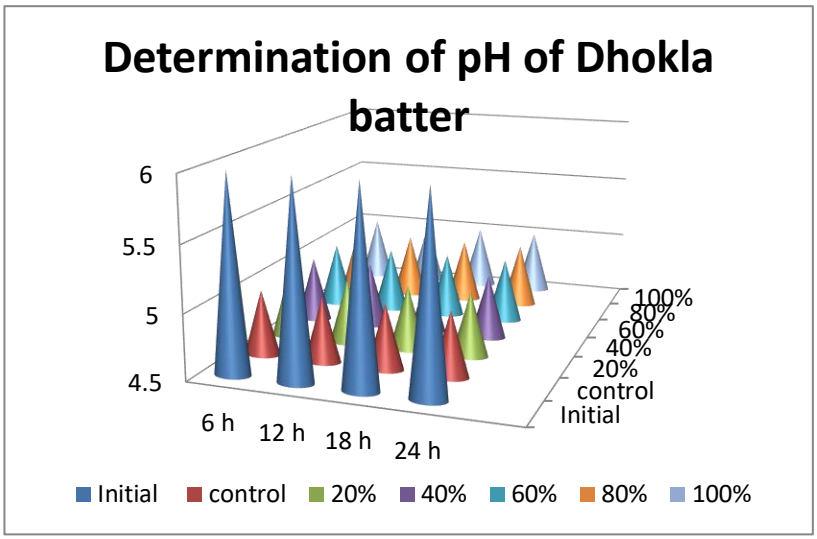

Figure 3.3: pH of Dhokla batters

3.5.3 Volume:

Initial volume of the batter was 40 ml for all the variations and increased gradually till 18 hours. At 24 hours the volume of batter was reduced. This increased volume of Dhokla batter is due to increase incorporation of the lactic acid bacteria into the batter during fermentation and entrapment of air.

Table 3.4: Determination of Volume of Dhokla batter

Variations	Initial volume (ml)	volume after 6 hrs (ml)	Volume after 12 hrs (ml)	Volume after 18 hrs (ml)	Volume after 24 hrs (ml)
P1 (control)	40	50	55	59	52
P2 20%	40	50	54	56	53
P3 40%	40	49	50	56	46
P4 60%	40	56	65	72	78
P5 80%	40	45	51	56	45
P6 100%	40	40	40	40	40

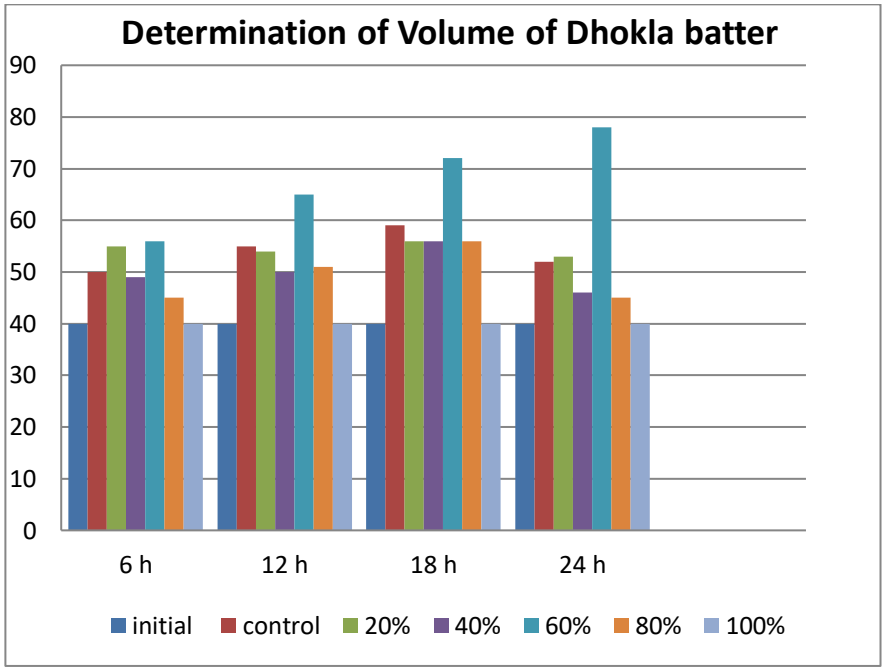

Figure 3.4: Volume of Dhokla batters

3.5.4 Optimization of Batter Fermentation:

The data pertaining to the effect of incubation of different fermentation time of Pearl millet grains (6 hrs. 12 hrs. 18 hrs. 24 hrs.). The sensory attributes of Dhokla and the results are shown in Table 3.5. The scores obtained for all sensory attributes of PM1, PM2, PM3 and PM4. The variation PM1 was more acceptable in terms of sensory attributes.

Table 3.5: Sensory evaluation of different variation of Dhokla developed from Pearl millet with partial replacement of Bengal gram dhal by varying fermentation time.

Attributes	PM1 (6 hrs)	PM2 (12 hrs)	PM3 (18 hrs)	PM4 (24 hrs)
Appearance	8.71 ± 0.12*	7.91 ± 0.6	7.21 ± 0.35	6.71 ± 0.5
Colour	8.42 ± 0.16	8.23± 0.4	7.92 ± 0.23	6.52 ± 0.5
Texture	8.62 ± 0.13	7.82 ± 0.23	7.23 ± 0.9	6.23 ± 0.6
Taste	8.52 ± 0.22	7.71 ± 0.36	7.29 ± 0.9	6.31 ± 0.5
Flavour	8.71 ± 0.32	7.51 ± 0.76	6.92 ± 0.8	5.92 ± 0.5
Overall acceptability	8.92 ± 014	7.61 ± 0.32*	7.12 ± 0.9*	6.12 ± 0.5*

Values are mean SD, p ≤0.05 (Holm Sidak method), n=20

Figure 3.5: Pearl millet incorporated Dhokla developed by varying fermentation time

3.6 Nutritional Analysis of Prepared Dhokla P3 (40%):

The proximate composition of acceptable Pearl millet dhokla (P3) and that of control were analyzed and the results of the same are shown in Table 3.6.

The moisture content of all the variations of Dhokla was similar. The values of protein, dietary fibre and calcium content was higher in P3 than that of standard, whereas carbohydrate was less. However, iron and Calcium content were increased in Pearl millet Dhokla.

Table 3.6: Nutritional composition of selected variation (P3) of Dhokla developed from Pearl millet with partial replacement of Bengal gram dhal.

Nutrients/100 g	Standard	P3 (40%)
Moisture (%)	19.06± 0.36	29.21 ± 0.31
Carbohydrates (g)	49.16± 0.15	38.22 ± 0.11
Protein (g)	12.18 ± 0.12	13.98 ± 0.21*
Fat (g)	6.32 ± 0.32	8.28 ± 0.16
Crude fibre (g)	0.55± 0.10	0.87 ± 0.23*
Ash (g)	2.83 ± 0.02	0.61 ± 0.12
Energy (kcal)	338.8 ± 0.42	318 ± 0.33
Iron (mg)	5.33 ± 0.13	6.01 ± 0.13*
Calcium (mg)	44.02 ± 0.72	53.01 ± 0.22*
Phosphorous (mg)	285 ± 0.28	275 ± 0.22

Values are mean ± SD (n=20), *p<0.05 (Holm Sidak method)

3.7 Conclusion:

Dhokla is a Pulse based fermented product made from fermented rice and Bengal gram dhal eaten for breakfast, fermentation increases folic acid, raise the batter volume and gives sponginess to the product. The role of the lactic acid bacteria is to reduce the pH of the batter to an optimum level for the yeast activity in the Dhokla batter. Standardization of fermentation time for selected variation resulted in more acceptability in terms of sensory attributes with maximum scores for PM1 (6 hrs.). Pearl millet Dhokla of 40% (P3) had highest acceptability in terms of sensory scores next to P2 whereas, P5 (80%) & P6 (100%) had least acceptability. As a result of poor fermentation, PM4 (24 hrs.) had lowest sensory scores and the last variation was also not good due to hyper-fermentation. Pearl millet Dhokla had increased level of protein, Dietary fiber, Iron, moreover it reduced the carbohydrate content making it low GI. The optimum fermentation time for Pearl millet incorporated Dhokla was found to be 6 hrs. and acceptable up to 40%.

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4. Development and Quality Evaluation of Bhathura from Kodo Millet (*Paspalum Scrobicutalum*)

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Abstract:

Fermentation is a natural process which reduces phytic acids and tannins, therefore enhancing the protein availability and digestion in millets. In Bhatura, Lactic acid bacteria and Yeast fermentation takes place. It improves the palatability and acceptability by developing improved flavours, textures and nutritive content. Bhatura is a fluffy deep-fried leavened sour dough bread originating from the Indian subcontinent. Kodo millet is very beneficial which are rich in antioxidants, dietary fibre, phenolic compounds and B Vitamins like niacin, folic acid, as well as the minerals such as iron, potassium and zinc. The potential health benefits include weight loss, improve gut health and bowel movement and helps in irregular menstrual cycles and reduces its cramps. This study was conducted to develop and evaluate the fermented Bhatura by replacing the Maida with Kodo millet of six different compositions K1, K2, K3, K4, K5 and K6 along with the semolina, baking soda, baking powder, salt, sugar and curd with different proportions of Kodo millet (0%, 20%, 40%, 60%, 80% and 100%). The developed products were analysed for its sensory attributes (n=20). Fermentation of the developed product was optimised and detected by performing variations in fermentation time (1- 4 h). The highest sensory score obtained for K3 (40%) which was fermented for 3 h. The proximate nutritional composition of the best accepted variation was analysed by standard procedures, which were high in energy, carbohydrate, fibre and iron when compared with standard Bhatura.

Keywords:

Fermentation, Lactic Acid Bacteria, Antioxidant.

4.1 Introduction:

Cereals and Legumes are a very good source of macronutrients, micronutrients, phytochemicals and anti-nutritional factors. Fermentation is a desirable process of biochemical modification of primary food matrix brought about by microorganisms and their enzymes (1). It is used to enhance the bio accessibility and bioavailability of nutrients from different crops and improves the organoleptic properties as well as extending the shelf life of the food product (2).

It makes food safe by not only inhibiting the growth of pathogenic bacteria due to antimicrobial activity of lactic acid, but also detoxifies the aflatoxin (3). In carbohydrates, fermentation activates starch- hydrolysing enzymes such as α- amylase and maltose which degrade starch into maltodextrins and simple sugars respectively (4).

In proteins, fermentation followed by cooking was effective in increasing the digestibility of grain protein (5). It also increases the bioavailability of calcium, iron and phosphorus due to the degradation of oxalates and phytates (6). The increase in mineral content might be due to the loss of dry matter during fermentation as microbes degrade carbohydrates and protein (7).

Bhatura's are the fried version of naan. It is a fluffy deep- fried leavened sourdough bread originating from the Indian subcontinent. Sour curd is added to dough in Bhatura's cooked by traditional method to reduce the fermentation time and consequently influence the microbial profile of the total fermentation process (8). Bhatura is a good source of energy and fat which is usually consumed with chole (9). Along with yeast, lactic acid bacteria aid the fermentation process which makes the Bhatura palatable as well as acceptable.

Millets are a good substitute to rice and wheat as they are gluten-free. Kodo Millet (*Paspalum scrobiculatum*), one of the ancient grains of the world which belongs to Poaceae family. It is also known as cow grass, rice grass and crown grass (10). Kodo millet is a nutritious grain, which is rich in protein, fibre and minerals. It is a rich source of antioxidants and polyphenolic compounds which is very beneficial to human health. It lowers blood glucose levels, blood pressure and exhibit anti-allergic and antibacterial properties (11).

Bhatura **Kodo Millet**

Figure 4.1: Bhatura and Kodo Millet

4.2 Traditional Fermented Foods:

Indigenous or traditional fermented foods have been prepared and consumed for hundreds of years and are strongly linked to cultures and traditions of millions of people around the world, especially in rural communities. Fermented food products are important components of the diet as staples, adjuncts to staple, condiments and beverages. Some of the Indian traditional fermented foods are Dhokla, Jalebi, Naan, Kinema, Wadi, Mesu and Bhatura (12).

4.3 Objective:

- To develop a Bhatura using Kodo Millet Flour (KMF).
- To evaluate the organoleptic properties of bhatura by varying the proportions of KMF
- To study the effect of fermentation time on sensory attributes of the product.

4.4 Materials and Method:

4.4.1 Raw Materials:

The present study was carried out in the department of Food Sience and Nutrition, Yuvaraja's college, (Autonomous) University of Mysore, Mysuru.

The raw materials such as Maida flour, Kodo millet flour, semolina, salt, sugar, curd, baking soda, baking powder and oil were procured from local market of Mysuru.

4.4.2 Method of Preparation:

Bhatura was prepared by adding different ratio of Maida flour and Kodo millet flour along with semolina, salt, sugar, baking soda and baking powder in a bowl. Mix the dry flours evenly, add hot oil and mix again. The curd is added to aid the fermentation and semi-hard dough was prepared.

The dough is kneaded properly and oil was smeared over the surface of the dough. The bowl is closed using the muslin cloth and it is kept for fermentation for about 2-3 hours. The balls were made using the dough and rolled it to thick, uniform Bhatura. Finally, it is deep fried until it turns golden brown colour and crispy and puffed Bhatura.

4.4.3 Sensory Analysis of Prepared Bhatura:

Sensory evaluation of the Bhatura was made to determine the sensory attributes like appearance, taste, texture, color, flavor and overall acceptability. The product was evaluated by taking average score of the 20 semi trained panelists by using 9-point hedonic scale (13).

4.4.4 Nutritional Information of Kodo Millet:

Standard A.O.A.C. (1980) method was used to determine the Nutritional composition of selected variation (K3) of pearl millet and control.

The moisture content was estimated by using hot air oven at 98 to 100˚ C, Protein content was estimated by determining total nitrogen content using standard Micro – Kjeldhal method, ash % were estimated by high temperature incineration using muffle furnace and fat content was estimated by the Soxhlet method.

The crude fibre content was estimated by crude fibre analyzer.

The carbohydrate content was obtained by subtracting from 100 with the sum of values of moisture, protein, fat and ash content per 100 g of the sample.

Minerals like Calcium, iron and phosphorous were analyzed using inductively coupled plasma mass spectrometry (ICPMS). These methods give a good precision and accuracy (14).

4.4.5 Statistical Analysis:

The samples were analyzed by triplicates.

The data obtained was analysed statistically using standard methods given by Snedecor and Cochran and by Duncan's multiple range test with the $p \leq 0.05$ consider to be significant (15).

4.5 Flow Chart:

Take Maida flour / KMF, Semolina, Salt, Sugar, Baking Soda & Baking Powder in a bowl

↓

Mix the dry flours evenly and add hot oil and mix again

↓

Add Curd to aid the Fermentation and make semi-hard dough

↓

Knead the dough, smear oil over its surface and close the bowl using muslin cloth

↓

Let it ferment for 2 / 3 Hrs

↓

Make equal size balls out of dough and roll it to thick, uniform Bhatura

↓

Deep fry, till it become golden brown, crispy, puffed Bhatura

Figure 4.2: Flow Chart for Preparation of Kodo Millet Bhatura

4.6 Formulation of Bhatura:

Table 4.1: Formulation of the product (ingredients g/100gm) for preparation of Kodo millet flour Bhatura.

Ingredients	K1 (Control)	K2 (20%)	K3 (40%)	K4 (60%)	K5 (80%)	K6 (100%)
Maida flour (g)	100	80	60	40	20	-
Kodo millet flour (g)	-	20	40	60	80	100
Semolina (g)	15	15	15	15	15	15
Salt (g)	2	2	2	2	2	2
Sugar (g)	2.5	2.5	2.5	2.5	2.5	2.5
Baking soda (g)	1.25	1.25	1.25	1.25	1.25	1.25
Baking powder (g)	1.25	1.25	1.25	.25	1.25	1.25
Curd (ml)	30	30	30	30	30	30
Oil (ml)	3	3	3	3	3	3
Water (ml)	40	40	40	40	40	40

Figure 4.3: Different variations of Bhatura developed from Kodo millet flour in comparison of maida flour Bhatura.

4.7 Optimization of Fermentation Time:

A food is considered fermented when one or more of their constituents have been acted upon by the selected microorganisms or their enzymes to produce a significant altered final product desirable for human consumption (15).

The fermentation takes place through yeast and Lactic Acid Bacteria (LAB), it occupies a central role in this process.

It enhances the shelf life and microbial safety, improve texture and contribute to the pleasant sensory profile of the product (16).

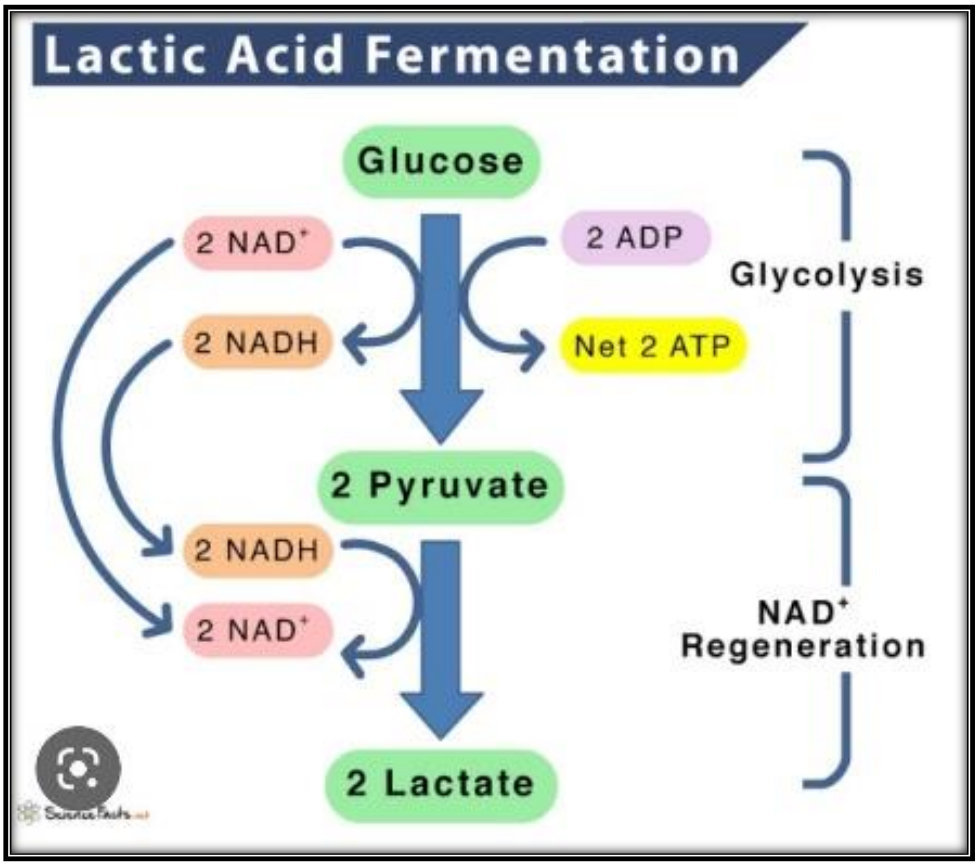

Figure 4.4: Lactic Acid Bacteria Process

The time, temperature, pH, relative humidity and oxygen availability during fermentation are the crucial factors responsible for the formation of high levels of desired products (17).

Of these, the time of fermentation is most important, since both increase and decrease in fermentation time can lead to the poor quality of Bhatura. Although some attempts had been made to show the effect of fermentation time on bhatura (18).

Figure 4.5: KMF incorporated Bhatura prepared by varying fermentation time.

4.8 Results and Discussion:

4.8.1 Sensory Evaluation of Kodo Millet Incorporated Bhatura:

This study aims to prepare the millet-based Bhatura by partially replacing the rice flour with Kodo millet flour.

The data shows the sensory attributes of Bhatura in effect of incorporation of Kodo millet flour with various levels [K2, K3, K4, K5 and K6] along with the control [K1] which are shown in Table 4.2.

The sensory score for K3 [40%] was highest with overall acceptability when compared to other variations.

Table 4.2: Sensory evaluation of different variation of Bhatura developed from Kodo millet flour with partial replacement of maida flour.

	K1 (Control)	K2 (20%)	K3 (40%)	K4 (60%)	K5 (80%)	K6 (100%)
Appearance	8.89±0.53	8.45±0.36	**8.05±0.49**	7.45±0.58	6.65±0.57	6.55±0.49
Colour	8.83±0.46	8.05±0.48	**7.63±0.66**	7.15±0.72	6.55±0.49	6.53±0.35
Texture	8.75±0.33	8.25±0.21	**7.90±0.83**	7.20±0.92	6.65±0.57	6.40±0.61
Flavour	8.75±0.33	8.8±0.57	**8.00±0.69**	7.20±0.50	6.75±0.69	6.67±0.82
Taste	8.68±0.56	8.40±0.43	**8.05±0.66**	7.05±0.65	6.75±0.62	6.54±0.75
Overall Acceptability	8.72±0.51	8.45±0.25	**8.00±0.63**	7.10±0.62	6.73±0.35	6.62±0.52

Values are mean ± SD, $p \leq 0.05$ (Holm Sidak method), n=20

4.8.2 pH of Kodo Millet Bhatura:

pH value of Bhatura dough at different fermentation period ranged from 6.0 to 7.0 (1, 2, 3 and 4 hours).

Initial pH was 7.0, as fermentation occurred the pH was 6.0 throughout process even after varying the fermentation time.

The acidity level was slightly increased i.e., decrease in the pH value of the Bhatura. This is mainly due to the presence of lactic acid bacteria which leads to the production of lactic acid that lowers the pH level and helps in leavening of the dough.

Table 4.3: pH of KMF Bhatura dough

Variations	Initial pH	pH at 1h	pH at 2h	pH at 3h	pH at 4h
K1 (Control)	7	6	6	6	6
K2 (20%)	7	6	6	6	6
K3 (40%)	**7**	**6**	**6**	**6**	**6**
K4 (60%)	7	6	6	6	6
K5 (80%)	7	6	6	6	6
K6 (100%)	7	6	6	6	6

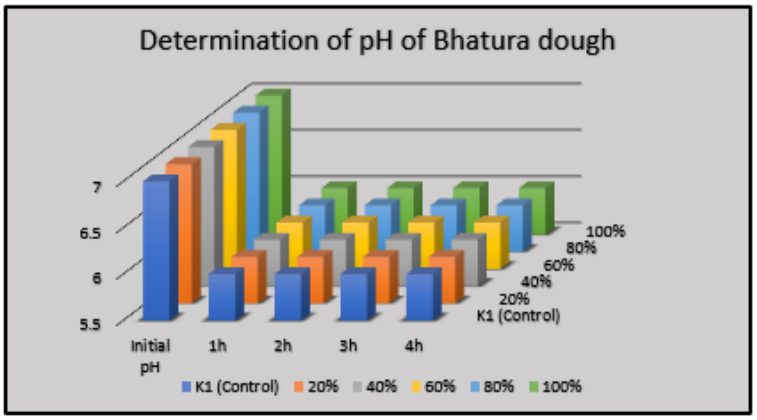

Figure 4.6: Determination of pH of KMF Bhatura dough

4.8.3 Optimization of Fermentation time of Dough:

The time was varied to know the effect of fermentation on Bhatura. The different time of fermentation are1h, 3h and 4h (K1, K2 and K4) where as standard bhatura was kept for 2 hours (K2) for fermentation as shown in Table 4.4. The sensory score for K3 [3h] was highest with overall acceptability when compared to other variations.

Table 4.4: Sensory evaluation of Bhatura developed from Kodo millet flour with different fermentation time.

	K1 (1h)	**K2 (2h)**	**K3 (3h)**	**K4 (4h)**
Appearance	7.45±0.49	7.30±0.45	**7.66±0.47**	6.78±0.89
Colour	7.45±0.49	7.50±0.67	**7.70±0.71**	6.55±0.73
Texture	7.25±0.43	7.52±0.59	**7.85±0.42**	6.66±0.79
Flavour	7.40±0.66	7.70±0.70	**8.10±0.62**	6.61±0.73
Taste	7.50±0.50	7.15±0.72	**7.95±0.49**	6.55±0.73
Overall Acceptability	7.50±0.50	7.30±0.64	**7.95±0.73**	6.68±0.86

Values are mean ± SD, $p \leq 0.05$ (Holm Sidak method), n=14

The Nutritional analysis was made to the selected variation [K3] and its control [K1] and the results are shown in Table.4.5. The macronutrients like energy, protein, and fibre content were more with less carbohydrate and fat. The significant difference can be seen in moisture level but not in ash. The micronutrients like iron and phosphorus were also more when compared with standard.

Table 4.5: Nutritional composition of selected variation (K3) of Bhatura developed from Kodo millet flour with partial replacement of Maida flour.

Nutrients	Standard Bhatura	KMF Bhatura
Energy (Kcal)	360.66 ± 0.45	363.94 ± 0.13*
Carbohydrate (g)	52.98 ± 0.33*	57.07 ± 0.57*
Protein (g)	8.27 ± 0.67*	5.76 ± 0.61
Fat (g)	25.9 ± 0.15*	22.53 ± 0.11
Crude fibre (g)	0.11 ± 0.58	0.37 ± 0.72*
Moisture (%)	10.88 ± 0.81	12.3 ± 0.49*
Ash (g)	1.86 ± 0.12	1.97 ± 0.28
Iron (mg)	1.74 ± 0.20	5.00 ± 0.08*
Phosphorus (mg)	1.90 ± 0.08	205 ± 0.89*

Values are mean ± SD, $p \leq 0.05$ (Holm Sidak method), n=20

4.9 Conclusion:

Fermentation increases the shelf life of the food product and enhances organoleptic properties. Bhatura is a puffy, leavened and deep-fried Indian bread. Kodo millet is a good source of antioxidants which lowers the blood glucose level and blood pressure. The Bhatura prepared with incorporation of Kodo millet flour was an innovative ideology. Standardization of fermentation time for selected variation resulted in more acceptability in terms of sensory attributes with maximum scores for K3 (3 h). Among all the formulations of Bhatura, K3 (40%) had highest sensory score, where K4 (60%), K5 (80%) and K6 (100%) had least acceptability. Proximate analysis of nutrients in selected variation of KMF Bhatura had significant increased level of protein, iron and Phosphorous content. Moreover, it reduced the carbohydrate content making it low GI. The optimum fermentation time for Kodo millet incorporated Bhatura was found to be 3 h and acceptable upto 40%.

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5. NEEM: A Traditional and Modern Medicine with Prominent Antibacterial Activity

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Abstract:

The neem tree, which has a long history of traditional use in Ayurvedic medicine, is well known for its therapeutic benefits. Neem has been used in traditional medicine for many years to cure a variety of conditions, such as bacterial infections, fungal infections skin problems, and digestive problems and many other diseases. This article gives a general review of the medicinal use of neem's active components particularly as antibacterial agents, and it's general phytochemistry, mechanism of action of active components and therapeutic role of neem in various diseases. Many Gram-positive and Gram-negative bacteria, including multidrug-resistant strains, have been discovered to be sensitive to the main bioactive components of neem, such as azadirachtin, nimbin, nimbidin, nimbolide and gallic acid. These compounds restrict bacterial growth and proliferation by acting on a variety of biological targets, including the production of cell walls, proteins, and DNA replication.

Neem exctracts have been shown to have immunomodulatory effects, enhancing the body's defense mechanism against bacterial infections. Antibacterial action of neem is confirmed by many researchers both in vitro and in vivo models and by clinical trials. In view of the global growth in antibiotic resistance, the therapeutic potential of neem compounds is particularly remarkable because it provides an alternative complement to traditional antibiotic therapy. Therefore, due to their well-documented efficacy, safety, and low toxicity profile, neem and its active components have significant potential for the development of novel antibacterial medicines. The antibacterial properties of neem have been recognized for centuries, and modern scientific research has continued to validate its traditional use as an effective treatment for bacterial infections. As a result, neem represents a possible option for future drug development to fight against many bacterial diseases.

Keywords: *Neem, Gram positive and Gram negative, Azadirachtin, Nimbin, Nimbidin, Nimbolide, Gallic acid, Antibacterial.*

5.1 Introduction:

Azadirachta indica, often known as Neem (family Meliaceae), since ancient times has been widely used as a source of therapeutic agent with active ingredients to create products for healthcare professionals in the far locations. The tree or its parts have been used in primary healthcare in impoverished nations. (1) People are now turning to old ethnomedical plant-based cures to prevent and treat illnesses as well as to preserve their health as a result of the rising cost of medicine in the modern era. Neem has been utilized as a medicinal plant for more than 2000 years. High biological activity makes it an adaptable therapeutic plant. (2)

In India, practitioners of Ayurvedic, Siddha, and herbal medicine frequently prescribe Neem tree components because of their well-known therapeutic qualities. Numerous qualities of neem leaf, include antibacterial, antiparasitic, anti-fungal, anti-inflammatory, and antioxidant. (3) The Neem tree has been used in traditional medicine for many years and is mostly grown in the southern parts of Asia and Africa. It's important to note that several Neem tree elements, such as leaves, bark, fruit, flowers, oil, and gum, are linked to the aforementioned medicinal folklore in the treatment of disorders like cancer, hypertension, heart disease, and diabetes. Cellular and molecular mechanisms, such as those involved in free radical scavenging, detoxification, DNA repair, cell cycle alteration, programmed cell death mitigation, autophagy, immune surveillance, anti-angiogenic, and anti-metastatic activities, and the capacity to modulate different signalling pathways, are undoubtedly responsible for the potential effects that may be observed when using these extracts. (4)

Azadirachta indica contains a variety of different elements, including as nimbin, nimbidin, nimbolide, and limonoids. These kinds of chemicals are useful for managing disorders by altering various genetic pathways and other processes. The first polyphenolic flavonoids to be isolated from freshly harvested neem leaves were quercetin and ß-sitosterol, which were also recognized to have antifungal and antibacterial properties. There have been numerous reports of biological and pharmaceutical activities, such as antibacterial, antifungal, and anti-inflammatory effects. Their role as anti-inflammatory, anti-arthritic, antipyretic, hypoglycemia, anti-gastric ulcer, antifungal, antibacterial, and antitumor actions have been proven by earlier researchers. (5)

Neem has historically been used to treat ailments that purify the blood and skin. Neem's impact on the skin is possibly its most lauded benefit. As general antiseptics, preparations made from the tree's leaves or oils are employed. Neem is excellent in treating most epidermal disorders such acne, psoriasis, and eczema because of its antibacterial qualities. Neem's bitterness was thought to balance out the sweetness, which was thought to be the cause of skin problems according to ancient ayurvedic practitioners. Indians used to have hot water with neem leaves steeped in it as their bath. Topical application of neem has been used frequently to treat skin conditions and allergic reactions because there have been no reports of any negative side effects.

Neem, especially when applied directly to the skin, has the potential to treat warts, chicken pox, and smallpox with antiviral effects. (8) This article provides an overview of how neem and its active constituents can prevent and treat disease by altering a number of biological processes.

5.2 Taxonomic Classification of Neem:

Botanical Name *Azadirachta indica*

Family belongs to Meliaceae Family

Common Names Nimba/Holy tree/Vembu/Arishta/Indian neem tree/Indian Lilac/Margosa tree.

Geographical source the neem tree is a fast-growing tree that can grow up to 15-20 m tall and has tiny, bright green leaves. It is abundant in tropical and semitropical areas. It blooms in the spring with a profusion of white flowers.

Plant Discription *Azadirachta indica* is a small to medium-sized tree that grows to a height of 15 to 30 metres and has a large crown that can reach 10 to 20 metres in circumference. (9) The leaves are 20–40 cm long, light green, and simple pinnate. Its taxonomical classification describes below. The bisexual, pentamerous, tiny, white or pale-yellow blooms have a faint sweetness to them. The plant's fruits are ellipsoidal, 1-2 cm long, greenish yellow to yellow or purple, and include one or two seeds. When ripe, they have ovoid or spherical seeds and are greenish in colour. (6) Neem's leaves, seeds, bark, roots, fruits, and oil have become the talk of contemporary medicine and are used medicinally to treat a variety of illnesses, particularly in Indian Ayurvedic, Homoeopathic, and Unani medicine. (7) Table: 5.1 provides the taxonomical classification of Neem.

Kingdom	**Plantae**
Phylum	Vascular plant
Class	Magnoliopsida
Order	Rutales
Suborder	Rutinae
Tribe	Melieae
Family	Meliaceae
Subfamily	Melioideae
Genus	*Azadirachta*
Species	*Indica*

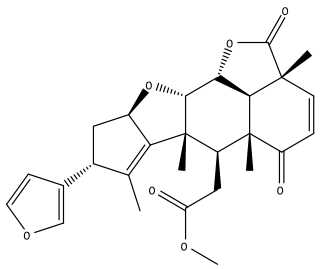
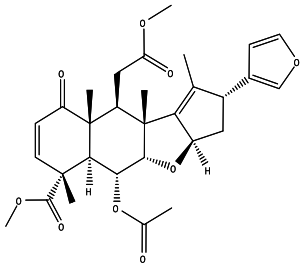
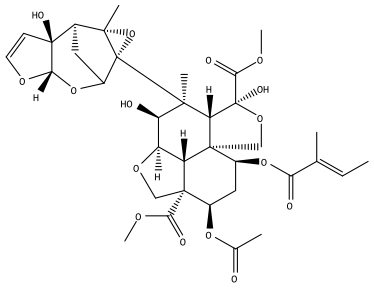
5.3 Active Constituents of *Azadirachta indica:*

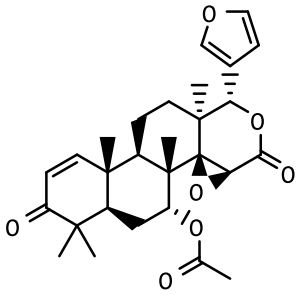
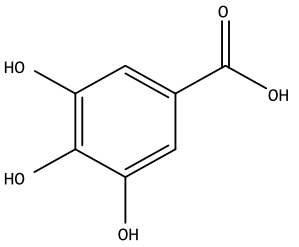
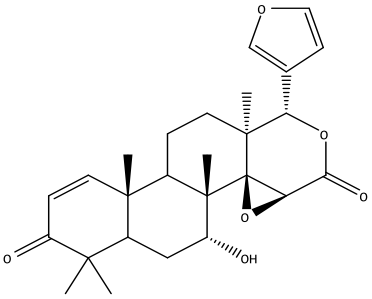
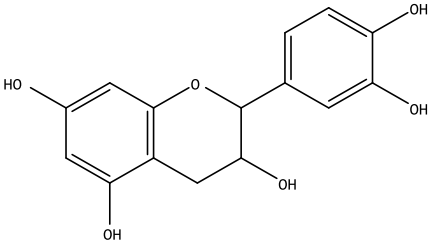
Neem, *Azadirachta indica*, plays a therapeutic role in the management of health because it is a rich source of many different kinds of ingredients. The most crucial active ingredient is Nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin are the other compounds in addition to azadirachtin. Ascorbic acid, n-hexacosanol, amino acids, 6-desacetylnimbinene, nimbandiol, nimbolide, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin,17hydroxyazadiradione, and nimbiol are among the compounds found in leaves.

The polyphenolic flavonoids quercetin and ß-sitosterol, purified from neem fresh leaves, were known to have antibacterial and antifungal properties, and seeds contain beneficial components like gedunin and azadirachtin.

Neem leaf extract (NLE) contains hydrocarbons, phenolic compounds, terpenoids, alkaloids, and glycosides [2-ethylhexyl tetradecyl est (13.70%), methyl petroselinate (11.23%), eicosane, 7-hexyl (10.01%), heptacosane (8.10%), hexadecamethylcyclo-octasiloxane Numerous investigations have demonstrated that terpenoids, particularly limonoids, are principally responsible for the biological effects of neem. (10) The majority of the active ingredients are tetranortriterpenoids, particularly azadirachtin analogues because they have antimicrobial and anti-infective properties. (7) Table 5.2 illustrates the major phytoconstituents present in Neem along with its chemical structure and uses.

Table: 5.2 Structures of major phytoconstituents of neem along with their uses

Phytoconstituents	**Structure**	**Use**	**Reference**
Nimbolide		Antimalarial, Antibacterial	(1)
Nimbin		Anti-inflammatory, Anti-arthritics, Hypoglycaemic, Antipyretic, Spermicidal, Antifungal, Antibacterial, Diuretic.	(7)
Azadirachtin		Antimalarial, Antibacterial	(7)

Phytoconstituents	**Structure**	**Use**	**Reference**
Gedunin		Antimalarial, Antifungal	(1)
Gallic Acid		Antibacterial	(11)
Limonoids		Anti-inflammatory' Antifungal	(1)
Catechin		Anti-microbial, Anti-viral and anti-cancer activities	(11)

5.4 Phytochemistry of Neem:

Triterpenes and limonoids are natural compounds found in neem. In neem, new limonoids are still being discovered.

Azadirachtin, salannin, meliantriol, and nimbin are well-known phytochemicals. Nimbin, the bitter constituent, contains an acetoxy, lactone, ester, methoxy, and aldehyde group. Sulphur is present in Nimbidin.

5.4.1 Flowers:

A flavonoid has been discovered in flowers. Nimbicetin is the same as kaempferol. The dried bark contains the same bitter components as the seed oil, and the fruit's pericarp contains the bitter principle bakayanin. (9)

5.4.2 Seed:

Gedunin, 7-desacetylgedunin, desacetylnimbin, and azedarachtin are meliacins found in the seeds. The seed oil is primarily composed of nimbidin, nimbin, and nimbinin, which are also found in the stembarkd.

5.4.3 Leaves:

Cyclic Trisulphide and Cyclic tetra sulphide, and also contains some triterpenes, and medicinally used in Leprosy, eye problem, epistaxis, intestinal worms, anorexia, biliousness, skin ulcers. (11), also contain Water, carbohydrates, protein, minerals, calcium, and phosphorus are all present in fresh, young neem leaves. Tyrosine, alanine, cysteine, glutamic acid, glutamine, and other types of amino acids and fatty acids are abundant in neem leaves. Antibacterial and anticancer properties can be found in neem leaf extracts and other parts. (12)

5.4.4 Bark:

A clear, bright amber-colored gum that is collected in tiny tears or fragments is exuded by the bark. Margosine is an unpleasant alkaloid found in it. In small amounts, leaves also contain bitter compounds that are much more soluble in water.

This substance is a resin hydrate. 10% to 31% of a yellow, bitter fixed oil with a strongly unpleasant, acrid flavour can be found in seeds. Stearic, oleic, and a trace amount of lauric acids make up the volatile fatty acids found in the bark.

Nimbin, Nimbinin, Nimbidin 0.4%, and Nimbidin 0.2% are all produced by the trunk bark. Along with tricyclic diterpenoids, tetracyclic triterpenoids and their derivatives have been isolated from the stem bark. (9)

5.4.5 Neem Oil:

Sulfur (0.427%), a very bitter yellowish substance derived from the oil's alcohol extract and thought to be an alkaloid, resins, glucosides, and fatty acids are all present in neem oil.

5.5 Mechanism of Action of Active Components:

Azadirachta indica has a therapeutic role due to its high concentration of antioxidants and other valuable active compounds like azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol, salannin, and quercetin.

Mechanism of action of *Azadirachta indica*: Parts of the neem (*Azadirachta indica*) plant exhibit an antimicrobial function by inhibiting microbial growth and/or the potential for cell wall breakdown. The main component causing both the antifeedant and toxic effects on insects is azadirachtin, a complex tetranortriterpenoid limonoid found in seeds. Results indicate that *Staphylococcus aureus* and *Methicillin-resistant Staphylococcus aureus* (MRSA) were both resistant to the ethanol extract of neem leaves in in vitro tests, with the largest zones of inhibition noted at 100% concentration. (13)

- Neem has the ability to scavenge free radicals because it contains a lot of antioxidants. In the following order: nimbolide > azadirachtin > ascorbate, nimbolide and azadirachtin both exhibited concentration-dependent antiradical scavenging activity and reductive potential.

Neem's ability to control cell signaling pathways makes it an effective cancer treatment ingredient. Neem controls the expression of several tumour suppressor genes, including p53 and pTEN, as well as angiogenesis (VEGF), transcription factors like NF-B, and apoptosis (e.g., bcl2, bax).

- Neem also functions as an anti-inflammatory by controlling the activity of proinflammatory enzymes like cyclooxygenase (COX) and lipoxygenase (LOX). (5)

5.6 Therapeutic Role of Neem in Various Diseases:

Figure 5.1 describes briefly about use of neem in different diseases. A brief outline is discussed below also.

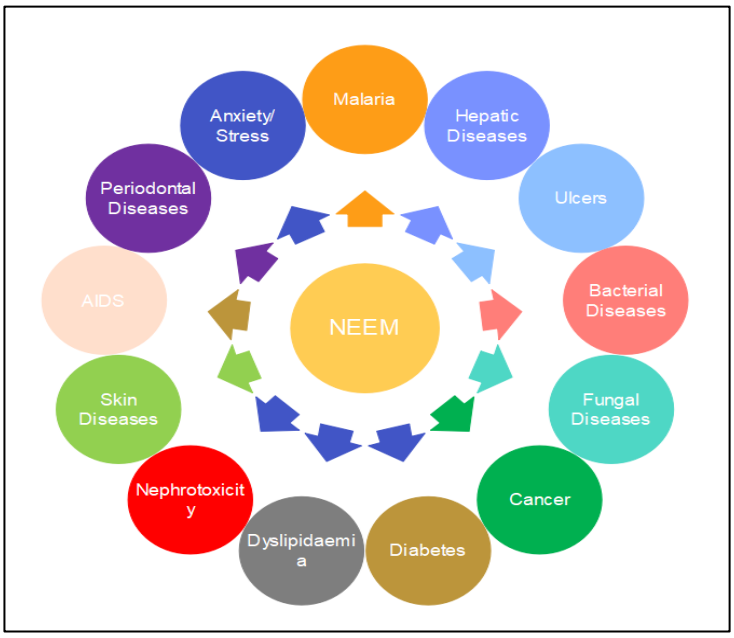


Figure 5.1: Therapeutic Role of Neem in Various Disease

5.6.1 Cancer:

Neem is effective in destroying cancer cells or enhancing the immune system to fortify it against harm. Neem or its isolated compounds have demonstrated impressive activity against a variety of human cancer cells, including those from the breast, prostate, colon, stomach, lung, and liver. In addition, some researchers have discovered that limonoid-derived chemicals have potent anti-cancer properties. Among them, it is worth noting that both 1-O-deacetylohchinolide B and 15-O-deacetylnimbolindin B have been shown to inhibit cell development in human cervical cancer by suppressing the NF-, Wnt / -catenin, and JAK / STAT pathways. (14)

5.6.2 Diabetes:

Neem has been used to treat disorders brought on by excessive sweet consumption due to its extremely bitter properties. Recent research has concentrated on the hypoglycemic properties of neem.

5.6.3 Topical Skin Diseases

Neem has proven to be extremely effective in treating a variety of skin conditions, including psoriasis, eczema, and other chronic conditions. Neem oil is effective in treating psoriasis. The skin loses colour in patches as a result of the autoimmune disorder vitiligo.

Four grammes of neem leaves are to be taken three times daily, ideally before each meal. The reversal of discoloration may be aided by the application of neem oil to the affected areas. (8)

5.6.4 AIDS:

According to the National Institutes of Health Neem extracts killed the AIDS virus, and AIDS treatments using these extracts have received patents. (15)

5.6.5 heart diseases:

A heart attack can be brought on by blood clots, high cholesterol, arrhythmic heartbeats, and high blood pressure, among other things. Its leaf extracts have inhibited irregular heart rhythms, slowed rapid or excessively high heartbeats, decreased clotting, lowered blood pressure and bad cholesterol, and reduced blood clotting.

5.6.6 Hepatic Diseases:

Neem leaf aqueous extract was discovered to provide defence against paracetamol-induced liver necrosis in rats. On administration of the neem leaf aqueous extract, it was discovered that the elevated levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transpeptidase (GGT) indicative of liver damage were significantly decreased. (16)

5.6.7 Bacterial Diseases:

Since some studies have conclusively demonstrated that neem extracts can be potentially useful to control some foodborne pathogens and other spoilage organisms, neem extracts are abundant in antimicrobial compounds.

Zones of inhibition have been discovered in NLEs, further demonstrating their antimicrobial properties, and the extract displayed noticeably larger zones of inhibition than 3% sodium hypochlorite. The extracts of the leaves and seeds were tested against various dermatophytes to determine their minimum inhibitory concentration (MIC) and minimum fungicidal concentration.

The outcome showed that the MIC of seed extracts for all the dermatophytes tested was 31 g/mL. Additionally, it was discovered that a seed extract concentration of 15 g/mL was sufficient to alter the tested organisms' growth patterns. (17) Fig: 5.2 refers to different phytoconstituents possessing antibacterial action of neem.

5.6.8 Fungal Diseases:

Neem has long been thought to be effective against some fungi that infect the human body. The athlete's foot fungus, which affects the skin, hair, and nails, the ringworm, which attacks the feet's skin and nails, the fungi that grow in the lungs, bronchi, and mucous membranes, as well as the normal mucous flora that can become out of control and cause lesions in the mouth (thrush), vagina, and other places, are some of the significant fungi that neem preparations have been found to Neem leaf and oil seed extracts are effective against a number of fungi, including Candida, Trichophyton, Epidermophyton, Microspor, Trichosporon, and others.

5.6.9 Ulcers:

Aqueous extracts of neem leaf and bark exhibit extremely potent antiacid secretory and antiulcer activity. Nimbidin significantly inhibited the development of gastric lesions brought on by acetylsalicylic acid, indomethacin, stress, or serotonin as well as duodenal lesions brought on by histamine or cysteamine. Nimbidin is known to have an antiulcer action, preventing acetylsalicylic acid, omethacin, serotonin-induced stomach and duodenal ulcers, and histamine. As a probable mechanism, neem leaf extracts exhibit antiulcer action, mucus depletion inhibition, and most cell defragmentation. The phenolic glycoside was identified as an active ingredient by the researchers. Furthermore, *Azadirachta indica* is a wonderful option for an active antiulcer and safe drug. (18)

5.6.10 Malaria:

Neem leaf and stem bark extracts significantly reduce parasitemia in infected mice by amounts of 51–80% and 56–87%, respectively. Additionally, other studies have shown that azadirachtin and other limonoids present in neem extracts have antimalarial activity against malaria vectors.

Another study using crude acetone and water (50/50) extract of leaves (IRAB) to analyse the activities against the sexual and asexual forms of Plasmodium falciparum in vitro found that, in separate 72-hour cultures of both mature gametocytes and asexual parasites treated with IRAB (0.5 microg/mL), parasite numbers were less than 50% of those in control cultures, which had 8.0% and 8.5% respectively. (3)

5.6.11 Periodontal Diseases:

Neem extracts prevent tooth decay and periodontal disease, which promotes good oral health, according to German researchers. *Candida albicans* and *Enterococcus faecalis* are both resistant to neem leaf extract's antimicrobial properties. As a result, it may have endodontic irrigant potential. (12)

5.6.12 Anxiety:

Neem leaf extracts in small doses have sedative properties. At high doses, roughly 400 or 800 milligrams per kilograms of body weight, the effect vanishes. It also lessens stress and anxiety.

5.6.13 Nephrotoxicity:

Nephrotoxicity, another name for renal toxicity, is primarily brought on by chemicals that interfere with the kidneys' normal operation. Neem has been shown in the literature to have protective effects against nephrotoxicity.

This claim has been supported by numerous studies on the kidney damage caused by cisplatin in wistar rats. Rats were divided into three groups for one study, which was conducted. Group one served as the control, Group 2 received an intraperitonial injection of 10 mg/kg cisplatin, and Group 3 received a 14-day treatment regimen that included 500 mg/kg/day of neem leaf extract in addition to the 10 mg/kg cisplatin. After drawing blood via cardiac puncture, serum creatinine, urea, and electrolytes were measured. The results demonstrated that pre-treatment with neem leaves extract normalised serum creatinine, urea, and electrolyte levels as well as reverted kidney apoptosis and necrosis. As a result, it can be said that neem leaf extract may be able to reduce nephrotoxicity while also preserving the kidney's normal function in rats. (2)

5.6.14 Anti-inflammatory Activity:

Methanolic extract of neem leaves possesses potent anti-inflammatory properties. This study demonstrated reduction of TNF-induced damage as well as suppression of nuclear factor (κB). Neem seed oil contains anti-inflammatory properties.

One study found that a combination of ethanolic extract of *A. indica* and Lawsonia Inermis leaf extract has strong anti-inflammatory effect at 2 mL per kilogramme body weight. (19) Table: 5.3 briefs about the major phytoconstituents of neem used in some diseases and the effects they cause.

Table: 5.3 Effects of different phytoconstituents of neem in various diseases

Disease	Name of the Phytoconstituents	Conclusion/Effect	References
Malaria	Azadirachtin, limonoids	Neem leaf and stem bark extracts significantly reduce parasitemia in infected mice by amounts of 51–80% and 56–87%, respectively. Additionally, other studies have shown that azadirachtin and other limonoids present in neem extracts have antimalarial activity against malaria vectors.	(3)
Hepatic	azadirachtin-A	The hepatoprotective role of azadirachtin-A in carbon tetrachloride (CCl4) induced hepatotoxicity in rats was investigated, and histology and ultrastructure data revealed that pretreatment with azadirachtin-A dosage dependently reduced hepatocellular necrosis.	(5)
Ulcers	Nimbidin	Nimbidin significantly inhibited the development of gastric lesions brought on by acetylsalicylic acid, indomethacin, stress, or serotonin as well as duodenal lesions brought on by histamine or cysteamine	(9)
Bacterial	Azadirachtin Nimbolide, Nimbidin Gallic acid, Mahmoodin Margolone	Neem extracts are high in antibacterial chemicals, and studies have shown that they can be used to inhibit some foodborne diseases and other spoilage organisms. NLEs were discovered to have zones of inhibition, demonstrating their antibacterial capabilities, and the extract had much higher zones of inhibition than 3% sodium hypochlorite.	(15)
Fungal	Limonoids' Gedunin	An experiment was conducted to evaluate the effectiveness of different neem leaf extracts on the seed-borne fungus	(3)

Disease	Name of the Phytoconstituents	Conclusion/Effect	References
	Cyclic Trisulphide and Tetrasulphide	Aspergillus and Rhizopus, and the results showed that both species' growth was greatly suppressed and controlled by the use of both alcoholic and water extract.	
Cancer	Azadirachtin nimbolide Catechin	An investigation was conducted to determine the chemo preventive potential of limonoids, azadirachtin, and nimbolide. The findings revealed that azadirachtin and nimbolide prevented the activation of procarcinogens and oxidative DNA damage, increased the activity of antioxidant and carcinogen-detoxification enzymes, and inhibited tumour invasion and angiogenesis.	(5)
Diabetes	liminoids Azadiradione	Neem leaves include phytochemicals including liminoids and azadiradione that are potent inhibitors of the human pancreatic alpha-amylase, decreasing postprandial glucose levels. These liminoids are thought to be the primary anti-diabetic target for regulating blood glucose level because they bind to and inactivate alpha-amylase.	(2)
Dyslipidaemia	Azadiractolide	Neem is also helpful in reversing dyslipidemia, according to a study, by lowering levels of triglycerides, low density lipoprotein, and VLDL while raising levels of HDL. The primary element in neem leaves that controls dyslipidemia is azadiractolide.	(2)
Nephrotoxicity	Nimbolide Nimbinene	A study was carried out to investigate the effect of neem leaf extract on cisplatin-induced hepatic and nephrotoxicity. Neem leaf extract significantly	(2)

Disease	Name of the Phytoconstituents	Conclusion/Effect	References
		reduced hepatic and renal damage by regulating serum urea, uric acid, creatinine, total bilirubin, ALP, AST, ALT, and -GT levels.	
Skin	Nimbidin Nimbolides Azadirachtin, Meliacin Salanin	This scientific evidence, supported by traditional use and literature, demonstrates the efficiency of Neem in the treatment of many skin ailments. Neem oil is an effective treatment for psoriasis. Vitiligo is thought to be an autoimmune condition that causes colour loss in regions of skin. The recommended dosage is four grammes of Neem leaves three times a day, ideally before each meal.	(20)
AIDS	Azadirachtin Nimboline	The findings of this study confirm the safety of acetone water neem leaf extract against HIV/AIDS and its significant effect on CD4+ cells, allowing it to be employed in the creation of multiple drug combination treatments for HIV/AIDS patients.	(2)
Periodontal	Nimbidin Mahmoodin Nimbolide	German researchers established that Neem extracts reduce tooth decay and periodontal disease, resulting in improved oral health. Enterococcus faecalis and Candida albicans are both killed by neem leaf extract.	(12)
Anxiety/Stress	Gallic acid, Epicatechin, Catechin	Neem leaf extracts have sedative properties at low dosages. In high doses, around 400 or 800 milligrams per kilogramme of body weight, the action is lost. It also helps with anxiety and stress.	(8)

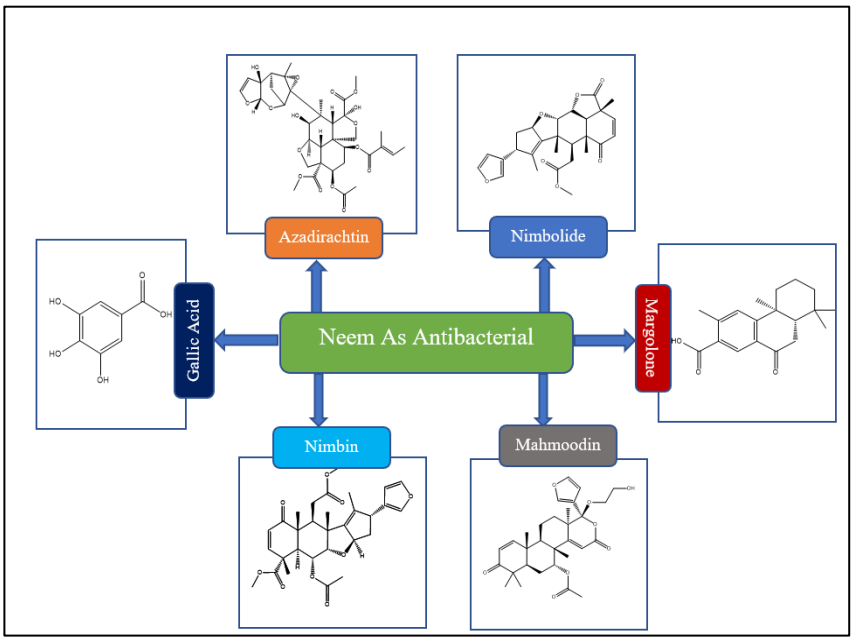


Figure 5.2: Phytoconstituents of Neem that Act as An Anti-Bacterial

5.7 Conclusion:

Azadirachta indica, tree can thrive in a range of soil types, including saline, alkaline, and acidic soils, and is well-adapted to arid and semi-arid conditions. Neem is one of the most adaptable medicinal plants in the world and has been used in traditional medicine for thousands of years. Neem is referred as “the healer of all ailments" or "Sarva Roga Nivarini" in the traditional Indian medical system of Ayurveda. In this review article we particularly focused on the antibacterial activity of neem with its active constituents mainly Azadirachtin, Nimbolide, Nimbin, Gallic acid, Mahmoodin, Margolone. Neem is also a well-liked component of traditional medical practices in various parts of the world, such as Southeast Asia and Africa. Biologically active substances, such as nimbin, nimbidin, nimbolide, azadirachtin, nimbolide, and quercetin in its leaves, bark, flowers, fruits, and seeds, have undergone substantial research for their potential therapeutic uses in treating a range of illnesses, including cancer, neurological diseases, gastrointestinal problems, skin disorders, and respiratory illnesses, and used in various homemade remedies.

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6. Development and Quality Evaluation of Dosa Prepared from Proso Millet (*Panicum Miliaceum*)

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Abstract:

Value added healthy foods are the requirement of modern society. Dosa is a thin pancake which is made from parboiled rice, black gram dhal, fenugreek seeds, salt and edible oil. It is a fermented product and it gives the characteristic texture, aroma, and taste to the Dosa along with increased digestibility and nutritional value. Lactic acid bacteria are mainly responsible for fermentation which are naturally present in the ingredients of Dosa batter, rice and black gram. Proso millet is rich in proteins, dietary fiber, polyphenols, vitamins and minerals and it is gluten free so ideal for gluten intolerance people. In the present study the attempt was made to develop Dosa by incorporation of Proso millet (P1, P2, P3, P4, P5, and P6) with parboiled rice at different incorporation levels. The levels are 0%, 20%, 40%, 60%, 80%, 100% were analysed for sensory attributes (n=15).

Developed product was acceptable up to 40% Proso millet in corporation. Its overall acceptability was 8.06±0.2 and nutritional composition of the best accepted variation were analysed by using standard procedures and observed that Proso millet Dosa is rich in moisture, minerals, phosphorus and iron. They have low glycemic index compared to traditional Dosa. Thus, acceptable product can be developed from Proso millet.

Keywords:

Microorganism, Fermentation, Lactic acid bacteria.

6.1 Introduction:

Fermentation is an ancient technique of preserving food. It is the chemical breakdown of a substances by the microorganisms typically involving effervescence and the giving of heat. It is a natural process which converts carbon dioxide such as starch and sugar into alcohol and acids and fermentation also promotes the growth of probiotic microorganisms, such as lactic acid bacteria. It has been shown to improve immune function as well as digestive and heart health. The main variable that are responsible for fermentation are mainly microorganisms and also the nutritional ingredients that present in the food and the environmental conditions [1]. Fermented food products have many benefits like it will increases the bioavailability of nutrients and stimulate probiotic and prebiotic functions.

It exerts vasodilatory action and provide beneficial effects against certain types of cancer and have beneficial effects on host gut mucosa like increase mucous production and enhance barrier integrity. It helps in the maintenance of homeostasis of Gastro Intestinal Tract. It ensures microbiological safety to the food. Fermentation will give a unique flavor to the food [2]. Dosa is one of the most popular fermented Indian dishes. It is a type of pan cake made from fermented batter of rice and black gram. According to Thankappan Nair, Dosa originated in the town of Udupi and according to food historian K.T Achaya references in the Sangam literature suggest that Dosa was already in use in the ancient Tamil country around the 1st century CE. Different varieties of Dosas are available in the market. Dosa has wide acceptance among the consumers [3]. Dosa is rich in carbohydrates, protein. Its main ingredients are Rice and Black gram dhal. Rice which is normally used for Dosa contains about 90% CHO, 8 % protein and 2 % fat. It is also good source of calcium, magnesium, phosphorus, manganese, selenium, iron and vitamin and Black gram dhal is rich in carbohydrates (60%), Proteins (25%), dietary fiber (18%) and good source of minerals, potassium, calcium, iron and vitamins. Found useful in controlling cholesterol levels [4]. Millets are nutritional crops and has various health benefits. Millets are rich in dietary fiber and which helps to lower blood glucose level and it binds to cholesterol and hinders its absorption and helps to protect from heart diseases. Slow fermentation of millets by micro flora promotes normal laxation which prevents constipation, diverticulosis and diverticulitis.

The scientific name of Proso millet is *Panicum miliaceum* and it belongs to the family Poaceae and is a self-pollinated crop. It is rich in phytochemicals and natural anti-oxidants. The proteins of Proso millet will reduce the risk of liver injury by suppressing the activity of D- galactosomine.

Being gluten free Proso millet is a great option for people with celiac disease which is a condition that the individual is highly resistant and allergic to gluten and Proso millet contain Lecithin which indirectly stimulates nervous system and make it to function properly [5]. In this study attempts are made to develop value added Proso millet (PM) Dosa. This product will get the benefits of both fermentation and Proso millet. The nutritional benefits of Proso millet have attracted attention and resulted in using Proso millet as food ingredient in preparing fermented food Dosa. Incorporation of Proso millet in Dosa batter will improves its efficiency.

Figure 6.1: Proso Millet (*Panicum Miliaceum*)

6.2 Objectives:

- To develop Dosa by partially replacing Rice with Proso millet.
- To evaluate the organoleptic acceptability of the developed products.
- To determine optimum fermentation time on the developed product.

6.3 Materials and Methods:

- **Raw Materials:** The present study was carried out in the department of Food Science and Nutrition, Yuvaraja's College, (Autonomous) University of Mysore, Mysuru. The raw materials such as Parboiled rice, Proso millet, Black gram dhal, Fenugreek seeds and salt were procured from local grocery shop of Mysuru.
- **Method of Preparation:** Dosa was prepared by Soaking Proso millet, Parboiled rice, black gram dhal and fenugreek seeds for 6 hours and it was ground by adding required amount of water and the batter were mixed well finally salt was added and allowed it to ferment for 12 hours at room temperature. Then the fermented batter was used to make Dosa.
- **Optimization of Batter Fermentation:** After addition of salt the batter was allowed to ferment for different period (6, 12, 18, 24 h) in a stainless-steel vessel. No effort was made to control the temperature during the process of fermentation.
- **pH and Volume:** For the different fermentation time and blend ratios of PM Dosa batter properties viz. Volume raised and pH were studied. The volume was recorded at 6, 12,

18 and 24 h with the help of measuring cylinder and pH of the batter at different fermentation time was determined by using pH paper before and after fermentation.

- **Sensory Analysis of Prepared Dosa:** Sensory evaluation was carried out to determine the acceptability of various attributes such as appearance, taste, texture, colour, flavour and overall acceptability. The product was evaluated by taking average score of the 15 semi trained panellists by using 9-point hedonic scale.
- **Nutritional Analysis of Prepared Dosa:** Standard A.O.A.C. (1980) method was used to determine the Nutritional composition of selected variation (P3) of Proso millet Dosa and control.

  By using hot air oven at 98 to 100˚ C the moisture content was estimated, Protein content of selected variation was estimated by determining total nitrogen content using standard Micro - Kjeldhal method, ash % were estimated by high temperature incineration using muffle furnace [6.7] and fat content was estimated by the Soxhlet method. The crude fiber content was estimated by crude fiber analyzer. The carbohydrate content was obtained by subtracting from 100 with the sum of values of moisture, protein, fat, fiber and ash content per 100 g of the sample.

  Minerals like iron and phosphorous were analyzed using inductively coupled plasma mass spectrometry (ICPMS). These methods give a good precision and accuracy [6].

- **Statistical analysis:** Each sample was analyzed in triplicates. The obtained data was analyzed statistically using standard methods given by Snedecor-- and Cochran [7] and by Duncan's multiple range test with the $p \leq 0.05$ consider to be significant [8, 9].

6.4 Formulation of the Product:

Table 6.1: Standardization of Formula ingredients (g/100gm) for preparation of PM Dosa

Ingredients	P1 (Control)	P2 (20 %)	P3 (40 %)	P4 (60 %)	P5 (80 %)	P6 (100 %)
Proso millet (g)	-	20	40	60	80	100
Parboiled rice (g)	100	80	60	40	20	-
Black gram dhal (g)	25	25	25	25	25	25
Fenugreek seeds (g)	5	5	5	5	5	5
Salt (g)	2	2	2	2	2	2

Proso millet, Parboiled rice, black gram dhal and fenugreek seeds were soaked for 6 hours

↓

Grind it by adding required amount of water

↓

Mix the batter well

↓

Add Salt

↓

Natural fermentation for 12 h at room temperature

↓

Fermented batter was used to make Dosa

Figure 6.2: Flow chart for preparation of PM Dosa

Figure 6.3: Different variations of Dosa developed from Proso millet in comparison with Standard Rice Dosa.

6.5 Results and Discussion:

6.5.1 Sensory Evaluation of PM Dosa:

The study was undertaken to prepare Dosa by partially replacing rice with Proso millet. The data pertaining to the effect of incorporation of various levels of Proso millet (20 %, 40 %,

60 %, 80 % and 100 %) on sensory attributes of Dosa and the results are shown in Table 6.2. The results showed that P3 (40 %) variation has most acceptability. The scores obtained for sensory attributes for P2 were almost similar on par with the control.

P5 showed decreased score and were less acceptable compared to other variations. The accepted P3 variation was incubated for different fermentation time to study the optimum fermentation time of PM incorporated Dosa.

Table 6.2: Sensory evaluation of different variation of Dosa developed from Proso millet with partial replacement of Rice.

Attributes	**Control (P1)**	**P2 (20 %)**	**P3 (40 %)**	**P4 (60 %)**	**P5 (80 %)**	**P6 (60 %)**
Appearance	8.60±0.50	8.13±0.4	**8.06±0.2**	7.3±0.4*	6.86±0.3*	7.4±0.5*
Colour	8.66±0.48	8.12±0.6	**8.12±0.5**	7.8±0.3	6.86±0.3*	7.33±0.5*
Texture	8.06±0.45	8.17±0.7	**8.20±0.4**	7.6±0.5	6.8±0.4*	7.06±0.5*
Taste	8.26±0.70	8.13±0.3	**7.8±0.5**	7.6±0.5*	6.8±0.4*	7.06±0.7*
Flavour	8.20±0.67	8.13±0.3	**8.06±0.2**	7.86±0.3	6.6±0.4*	7±0.7*
Overall acceptability	8.26±0.59	8.13±0.3	**8.06±0.2**	8±0.3	6.66±0.4*	7±0.5*

Values are mean ± SD, $p \leq 0.05$ (Holm Sidak method), n=15.

6.5.2 pH:

pH of the accepted P3 variation at different fermentation period was determined which ranged from 4.0 to 6.0 (6, 12, 18 and 24 hours). Initial pH was 6.0 as fermentation occurred pH was observed 6.0 at 6h and 5.0 throughout 12h, 18h and 4.0 at 24h.

There is an increase in acidity level i.e., decrease in the pH value. Acidic pH in Dosa is primarily due to the fermentation process.

Lactic acid fermentation is mainly responsible for the sour taste and acidic pH of Dosa batter. The lactic acid bacteria present in the batter converts the sugars present in the rice and lentils into lactic acid.

The acidic pH in Dosa batter is essential for the fermentation process. The acidic environment created by the organic acids inhibits the growth of harmful bacteria and promotes the growth of beneficial bacteria. It also contributes to the characteristic texture and taste of Dosa.

Table 6.3: pH of PM incorporated (P3) Dosa batter fermented for different time period

Variations (h)	Initial pH	pH after Fermentation
6	6	6
12	6	5
18	6	5
24	6	4

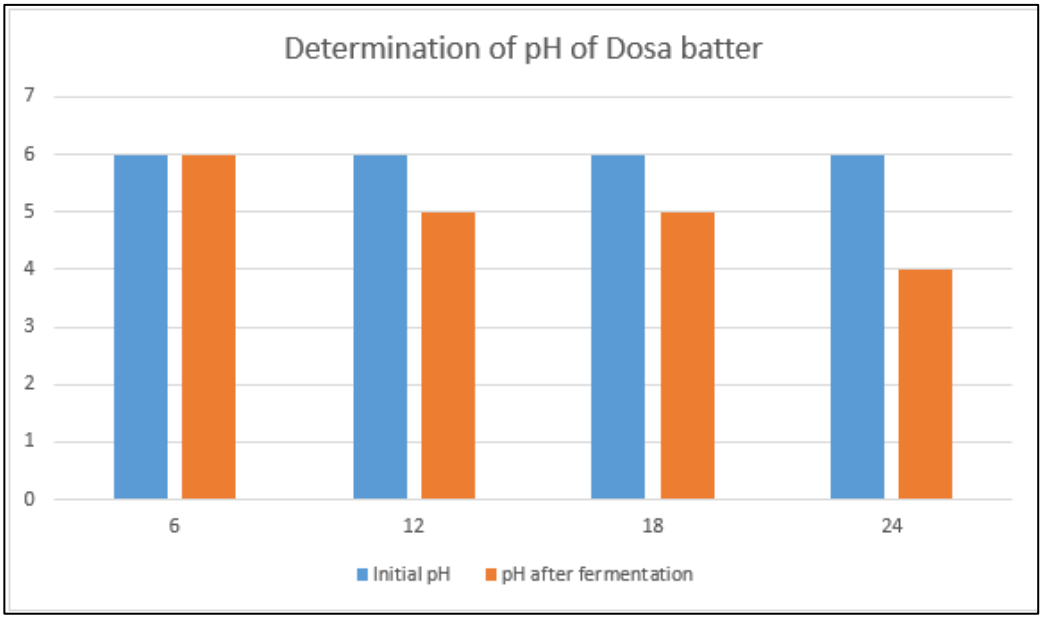

Figure 6.4: Determination of pH of PM incorporated Dosa Batter

6.5.3 Volumes:

Volume of accepted P3 variation at different fermentation time period was measured. The initial volume of the batter was 40 ml for all the variations and increased gradually till 24h. The volume rise is because of the production of carbon dioxide gas by the microorganisms present in the batter. The gas gets trapped within the batter, forming air pockets, and causing the batter to rise, resulting in the fluffy texture of Dosa.

Table 6.4: Volume of PM incorporated (P3) Dosa batter fermented for different time period

Variations (h)	Initial volume	Final Volume
6	40	48
12	40	50
18	40	55
24	40	59

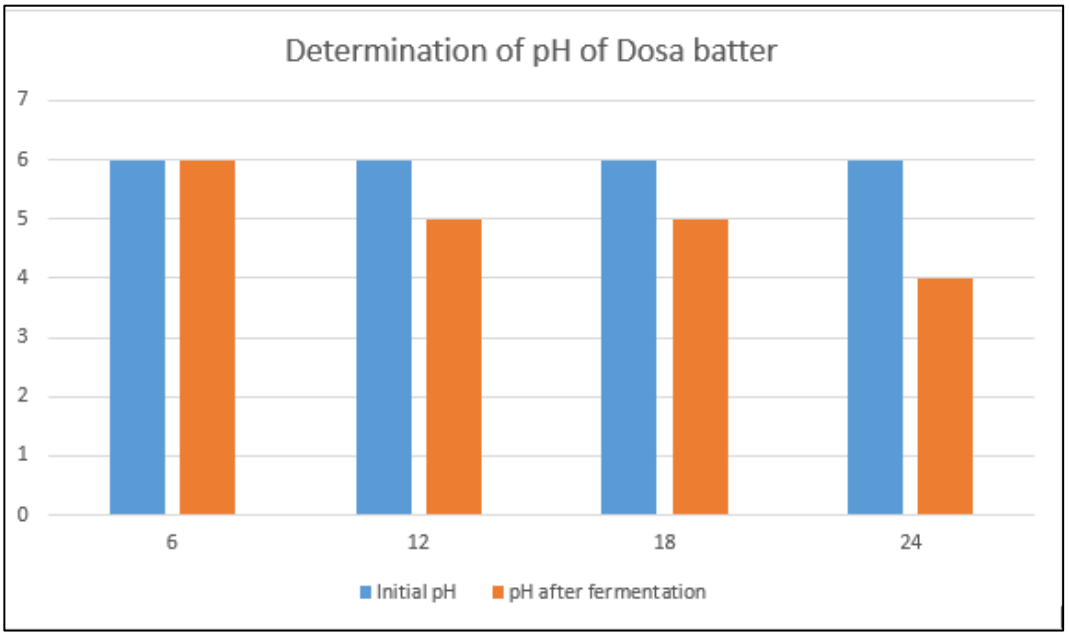

Figure 6.5: Determination of pH of PM incorporated Dosa batter

6.5.4 Optimization of Batter Fermentation:

The data pertaining to the effect of incubation of different fermentation time of PM grains (6 h, 12 h, 18 h, and 24 h).

The sensory attributes of Dosa and the result are shown in the table. The scores obtained for all sensory attributes of Control, PM 1, PM2, PM3, PM4. The variation PM 1 was more acceptable in terms of sensory attributes.

Table 6.5: Sensory evaluation of different variation of Dosa developed from PM with partial replacement of Rice by varying fermentation time.

Attributes	**Control**	**PM 1 (6 h)**	**PM 2 (12 h)**	**PM 3 (18 h)**	**PM 4 (24 h)**
Appearance	8.60±0.50	**8.26±0.5**	7.8±0.4	7.53±0.6*	7.2±0.5*
Colour	8.66±0.48	**8.06±0.7**	7.46±0.6*	7.46±0.6*	7.2±0.5*
Texture	8.06±0.45	**8.06±0.7**	7.86±0.7	7.53±0.9*	7.33±0.6*
Taste	8.26±0.70	**8.89±0.5**	7.93±0.7	7.13±0.9*	7.13±0.5*
Flavour	8.20±0.67	**7.93±0.5**	7.86±0.5	7.06±0.8*	7.13±0.5
Overall acceptability	8.26±0.59	**7.93±0.4**	7.86±0.6	7.13±0.9	7.13±0.5*

Values are mean ±, n=15 *p < 0.05 (Holm sidak method)

Figure 6.6: PM incorporated Dosa Developed by Varying Fermentation Time

6.5.5 Nutritional analysis of Prepared Dosa P3 (40 %):

The Proximate composition of acceptable PM Dosa (P3) and that of control were analyzed and the result of the same are shown in the table 6.6. Carbohydrate content of PM Dosa is less compared to standard Dosa. The moisture, minerals, Iron and phosphorus content was higher in P3 than that of control.

6.5.6 Nutritional analysis of prepared Dosa:

Table 6.6: Nutritional Composition of selected variation (P3) of Dosa developed from Proso millet with partial replacement of Rice.

Nutrients/100 g	Control	P3 (60 %)
Moisture (%)	20.05±0.15	26.88±0.07*
Carbohydrates (g)	57.7±1.08	55.72±0.19*
Proteins (g)	18.82±0.03	13.28±0.09*
Fat (g)	1.04±0.25	1.32±0.02*
Crude Fiber (g)	0.74±0.01	0.67±0.25*
Ash (g)	1.65±0.37	2.13±0.04*
Energy (kcal)	315.44±0.27	287.88±0.27*
Iron (mg)	3.69±0.02	4.45±0.64*
Phosphorus (mg)	257.75±0.05	282.95±0.02*

Values are mean ± SD, $p \leq 0.05$ (Holm sidak), n=3.

6.6 Conclusion:

Dosa is an Indian traditional cereal based fermented product. In this study the new product developed was Proso millet Dosa (PM Dosa). Dosa was chosen for this product development was because of the wide acceptance of Dosa among the consumers.

Organoleptic evaluation of Dosa revealed that P3 (40 %) with 6h fermentation time had high acceptance for its appearance colour, texture, flavor and taste among all. Fermentation helps in increased digestibility and nutritional value.

Lactic acid bacteria are mainly responsible for fermentation which improves the characteristic texture, aroma and taste to the Dosa. Millets are nutritionally superior crops. They are rich in minerals, Iron and proteins. It is gluten free so ideal for gluten intolerance people. Proso millet Dosa showed that it is highly acceptable to develop because of its improved sensorial characteristics, nutritional and health benefits.

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7. Anti-Microbial Activity of Madhu Tulasi (*Stevia Rebaudiana*)

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Abstract:

Stevia rebaudiana (SR) is a perennial shrub, belongs to Asteraceae family, popularly known as Madhu Tulasi. It is native to Paraguay and it is known for its sweetness and calorie free property. Madhu tulasi has pharmacological and therapeutic properties which include anti-microbial, anti-fungal, anti-oxidant, anti-carcinogenic, anti-septic, anti-diabetic, anti-inflammatory, diuretic and cardio-protective properties. The antimicrobial property is examined by extracting SR leaves in different components such as aqueous, methanol, ethanol, ethyl acetate, acetone, petroleum, chloroform, cyclohexane and hexane then testing it against few selected microorganisms like S. mutans, B. subtilis, S. aureus, M. luteus, S. marcenscens, P. aeruginosa, B. megaterium, E. coli, P. vulgaris, Yeast, A. niger and R. oligoporrus. The efficiency of these leaf extracts was compared to standard antibiotics like

tetracycline and streptomycin. Hexane extract showed highest zone of inhibition against E. coli. Whereas Aqueous extract of showed lowest zone of inhibition for E. coli growth. Hexane and ethyl acetate had greater inhibition effect compared to aqueous extracts. SR extracts had both anti-bacterial and anti-fungal effects. From this observation it is investigated that different extracts of SR leaves had anti-microbial activity on several microorganisms.

Keywords:

Madhu Tulasi, zone of inhibition, leaf extracts, microorganisms, Anti-microbial.

7.1 Introduction:

Stevia rebaudiana is a sweetener herb belong to the *Asteraceae* family, it is native to Argentina, Brazil and Paraguay. It is also known as sweet weed or honey leaf due to its high range of sweet content with zero-calorific value. *Stevia* is 100% natural flavour enhancer and non fermentable (1).

Traditionally SR is used to treat cough and to maintain oral health (2). Stevioside a natural sweetener extracted from leaves of *stevia* is composed of a mixture of several closely related ent-kaurene glycosides, among which rebaudioside A and dulcoside A and B are the most important. Longipinanes are the other phytochemical group commonly found in *Stevia*.

They are tricyclic sesquiterpenes that are frequently poly-esterified. These nutritional substances affect microbial flora of mouth (3). *Stevia* has many properties such as anti-fungal, anti-bacterial, anti-oxidant, anti-microbial, anti-inflammatory, anti-diabetic, hypertensive and hyper-glycaemic (4). Due to these immense beneficial properties, it is used worldwide as both sweetener and a medicinal herb. Consumption of stevia has health benefits such as controlling or managing metabolic disorder like diabetes. Phytochemicals such as terpenes, flavonoids and quinones in *Stevia* leaves are responsible for its anti-microbial activity.

7.2 Morphology of SR:

Table 7.1: Represents Morphology of SR (5)

01	Leaf	Leaves are small, sessile lanceolate to oblanceolate, oblong, serate above the middle and folded upwards. Trichomes on the leaf surface are of two distinct sizes, large [4-5 um] and small (2.5 um)
02	Seed	Seeds are contained in slender achenes about 3 mm in length. Each achene has about 20 persistent pappus bristles
03	Flower	The flowers are small (15-17 mm) and white with pale purple throat corollas. Anthers are small; five in number, the pollen can be highly allergic. Stigma id bi-lobed / bifurcated from the middle and style is surrounded by anthers.

7.3 Phytochemical Constituents of SR:

A renewed interest has occurred in the last decade to search for antibacterial acytivity and phytochemicas of native plants (6). Stevia contains over 100 phytochemicals (8). The sweetner, stevioside extracted from plants is 300 times sweetner than sugar (8). Stevioside is composed of a mixture of several closely related glycosides, among which stevioside, rebaudioside A and dulcoside A and B are the most important (9, 10, 11). Longipinanes are the other phytochemical group commonly found in *Stevia*. They are tricyclic sesquiterpenes that are frequently poly-esterified. This phytochemical group is the subject of numerous studies due to the extensive biological activities presented.

Table 7.2: Phytochemical Constituents of SR

Sr. No	Class of compounds	Compounds
01	Glycoside	Stevioside and rebaudioside A
02	Phenolic	Chlorogenic acid
03	Longipinanes	Tricyclic sesquiterpenes, germacranolides
04	Esters	Hydroxycinnaic acid, angelate
05	Flavones	Luteolin
06	Flavonols	Kaempferol, quercetin

7.4 Nutritional Composition of SR:

Dried SR leaves of 100g contains about 14.89 g of crude fibre, 31 g of carbohydrate, 19 g of protein, 6.7% of moisture, 11.5% of ash and minerals like phosphorous, sodium, potassium, calcium, iron, magnesium and chloride of 305mg, 184.3 mg, 2500 mg, 534.43 mg, 34.2 mg, 465.35 mg and 49.5 mg respectively. Thus, *Stevia* leaves are rich in protein and minerals such as calcium, phosphorous and magnesium.

7.5 Anti-Microbial Activity of SR:

Anti-microbial is a property which has the capacity to inhibit the growth of microorganisms. In this study different extracts of SR had taken to know their efficiency to inhibit the growth of microorganisms.

- The study conducted by Maryam et al., on *Streptococcus mutans* with Acetone extract, ethanol extract and methanol extract of 100 mg/ml concentration, the zone of inhibition observed was 28.7 mm, 28.0 mm and 21.3 mm respectively. The standard antibiotic tetracyclin of 10 mg/ml, showed 10 mm zone of inhibition.
- Sumit Ghoshal and others performed the study on different bacteria and fungi with several extracts such as petroleum extract, cyclohexane extract, chloroform extract, aqueous extract, acetone extract and ethanol extract of 250 µg/ml concentration. The standard antibiotic taken for bacteria was cotrimazolle (10 µg/ml) and for fungi was streptomycin (10 µg/ml). For *E. coli* aqueous extract showed maximum inhibition of 11

mm, *p. aerogenosa* got maximally inhibited by petroleum extract of 11 mm. petroleum extract also showed maximum inhibition of 16.3 mm foe *S. aureus*. While for *B. subtilis* acetone extract showed effective inhibition of 10.3 mm. In fungi such as *A. niger, P. chrysogenum, A. solani and H. solani* the maximum inhibition was also shown by petroleum extract only, which was 16 mm, 14 mm, 16 mm and 14 mm respectively. In this study petroleum extract was more effective compared to other extracts if *Stevia* leaves.

- Manish B Tadhani et al., used 50 mg/ml concentration of each aqueous extract, methanol extract, ethyl acetate extract and hexane extracts of *Stevia* leaves and tested it on several bacteria and fungi. Ciproflaxin (10 µg/disc) was the standard antibiotic taken for bacteria, while Amphotericin (100 units/disc) was taken as standard antibiotic for fungi.

  Hexane extract inhibited *E. coli* of 16.67 mm, which was maximum compared to other extracts. While *E.* coli was resistant to aqueous extract. Similarly, hexane extract showed maximum inhibition to *P. aerogenosa, S. aureus, M. luteus, A. niger, yeast* and *R. oligoporous* of 19.67 mm, 15.33 mm, 13.67 mm, 16.33 mm, 35.33 mm and 22.00 mm respectively. In case of *B, subtilis* ethyl acetate extract showed maximum inhibition of 9.99 mm. In this study aqueous extract was least effective whereas hexane extract was highly effective in inhibiting the growth of microorganisms.
- Sathishkumar Jayaram and others studied on anti-microbial activity of *Stevia rebaudiana*. Ethyl acetate extract, acetone extract, aqueous extract and chloroform extract of *Stevia* of 50 mg/ml concentration was taken. The tested microorganisms taken was *E. coli, S. aureus, B. subtilis, S. typhi, A. hydrophila* and *V. colerae*.

For *E. coli*, ethyl acetate extract and acetone extract effectiveness were same and the zone if inhibition was 10 mm for both the extracts, while aqueous extract did not show any zone of inhibition to *E. coli*. For *S. aureus* 19 mm of maximum zone of inhibition was by acetone extract, followed by ethyl acetate extract of 10 mm zone of inhibition. Whereas aqueous extract and chloroform extract was ineffective for *S. aureus.* Acetone extract showed a maximum inhibition of 18 mm for *B. subtilis,* and aqueous extract was inefficient. For *S. typhi* and *A. hydrophila* the maximum inhibition zone was observed by acetone extract which was 13 mm and 14 mm respectively. *S. typhi*was resistant to AQE, whereas *A. hydrophila* was resistant to both aqueous extract and chloroform extract. *V. colerae* was high susceptible to ethyl acetate extract with a zone of inhibition of 18 mm, while resistant to aqueous extract of *Stevia rebaudiana* leaves.

Table 7.3: Anti- microbial activity of Stevia rebaudiana [ACE-Acetone extract, ETE-Ethanol extract, MTE- Methanol extract, PEE- Petroleum extract, CHE- Cyclohexane extract, CHLE- Chloroform extract, AQE- Aqueous extract, HE- Hexane extract, ETAE-Ethyl acetate extract]

Sr. No	Model	Extract	Zone of inhibition in mm	Reference
1	*Streptococcus mutans*	• Tetracyclin (10mg/ml) • ACE (100mg/ml) • ETE (100mg/ml) • MTE (100mg/ml)	10 28.7 28.0 21.3	(12)

Sr. No	Model	Extract	Zone of inhibition in mm	Reference
2	*Escherichia coli*	• Cotrimazole(10µg/ml) • PEE (250µg/ml) • CHE (250µg/ml) • CHLE (250µg/ml) • AQE (250µg/ml) • ACE (250µg/ml) • ETE (250µg/ml)	22 7 5 7 11 10 5	(13)
		• Ciproflaxin(10µg/disc) • AQE (50mg/ml) • MTE (50mg/ml) • ETAE (50mg/ml) • HE (50mg/ml)	23.33 - 8.67 10.33 16.67	(14)
		• ETAE (50mg/ml) • ACE (50mg/ml) • AQE (50mg/ml) • CHLE (50mg/ml)	10 10 - 6	(15)
3	*Pseudomonas aeroginosa*	• Cotrimazole(10µg/ml) • PEE (250µg/ml) • CHE (250µg/ml) • CHLE (250µg/ml) • AQE (250µg/ml) • ACE (250µg/ml) • ETE (250µg/ml)	25 11 7 5 5 6 8	(13)
		• Ciproflaxin(10µg/disc) • AQE (50mg/ml) • MTE (50mg/ml) • ETAE (50mg/ml) • HE (50mg/ml)	27.00 - 11.00 12.00 19.67	(14)
4	*Staphylococcus aureus*	• Cotrimazole(10µg/ml) • PEE (250µg/ml) • CHE (250µg/ml) • CHLE (250µg/ml) • AQE (250µg/ml) • ACE (250µg/ml) • ETE (250µg/ml)	23 16.3 9 11 9.3 5 5	(13)

Sr. No	Model	Extract	Zone of inhibition in mm	Reference
		• Ciproflaxin(10µg/disc) • AQE (50mg/ml) • MTE (50mg/ml) • ETAE (50mg/ml) • HX (50mg/ml)	19.33 9.33 8.33 12.33 15.33	(14)
		• ETAE (50mg/ml) • ACE (50mg/ml) • AQE (50mg/ml) • CHLE (50mg/ml)	10 19 - -	(15)
5	*Bacillus subtilis*	• Cotrimazole(10µg/ml) • PEE (250µg/ml) • CHE (250µg/ml) • CHLE (250µg/ml) • AQE (250µg/ml) • ACE (250µg/ml) • ETE (250µg/ml)	22.5 10 5 5 9 10.3 5	(13)
		• Ciproflaxin(10µg/disc) • AQE (50mg/ml) • MTE (50mg/ml) • ETAE (50mg/ml) • HE (50mg/ml)	33.67 8.33 9.00 9.99 8.67	(14)
		• ETAE (50mg/ml) • ACE (50mg/ml) • AQE (50mg/ml) • CHLE (50mg/ml)	11 18 - 8	(15)
6	*Aspergillus niger*	• Streptomycin(10µg/ml) • PEE (250µg/ml) • CHE (250µg/ml) • CHLE (250µg/ml) • AQE (250µg/ml) • ACE (250µg/ml) • ETE (250µg/ml)	23 16 5 5 11 5 9	(13)
		• Amphotericin(100units/disc) • AQE (50mg/ml) • MTE (50mg/ml) • ETAE (50mg/ml) • HE (50mg/ml)	14.00 - 10.00 8.33 16.33	(14)
7	*Penicillium chrysogenum*	• Streptomycin(10µg/ml)	22 14	(13)

Sr. No	Model	Extract	Zone of inhibition in mm	Reference
		• PEE (250µg/ml) • CHE (250µg/ml) • CHLE (250µg/ml) • AQE (250µg/ml) • ACE (250µg/ml) • ETE (250µg/ml)	8 7 10 5 5	
8	*Alternaria solani*	• Streptomycin(10µg/ml) • PEE (250µg/ml) • CHE (250µg/ml) • CHLE (250µg/ml) • AQE (250µg/ml) • ACE (250µg/ml) • ETE (250µg/ml)	11 16 5 5 6 7 5	(13)
9	*Helmemthosporium solani*	• Streptomycin(10µg/ml) • PEE (250µg/ml) • CHE (250µg/ml) • CHLE (250µg/ml) • AQE (250µg/ml) • ACE (250µg/ml) • ETE (250µg/ml)	10 14 5 9 8 5 5	(13)
10	*Micrococcus luteus*	• Ciproflaxin(10µg/disc) • AQE (50mg/ml) • MTE (50mg/ml) • ETAE (50mg/ml) • HE (50mg/ml)	12.33 - 8.67 8.67 13.67	(14)
11	*Sacharomyces cervisiae*	• Amphotericin(100units/disc) • AQE (50mg/ml) • MTE (50mg/ml) • ETAE (50mg/ml) • HE (50mg/ml)	14.33 - 8.33 10.00 35.33	(14)
12	*Rhizopus oligosporus*	• Amphotericin(100units/disc) • AQE (50mg/ml) • MTE (50mg/ml) • ETAE (50mg/ml) • HE (50mg/ml)	15.00 - 8.67 11.00 22.00	(14)
13	*Salmonella typhi*	• ETAE (50mg/ml) • ACE (50mg/ml) • AQE (50mg/ml) • CHLE (50mg/ml)	11 13 - 7	(15)

Sr. No	Model	Extract	Zone of inhibition in mm	Reference
14	*Aeromonas hydrophila*	• ETAE (50mg/ml) • ACE (50mg/ml) • AQE (50mg/ml) • CHLE (50mg/ml)	11 14 - -	(15)
15	*Vibrio colerae*	• ETAE (50mg/ml) • ACE (50mg/ml) • AQE (50mg/ml) • CHLE (50mg/ml)	18 10 - 6	(15)

7.6 Conclusion:

In this study it was observed that, the susceptibility of microorganisms was different to each and every extract. Among these extracts, efficiency in inhibiting the microorganism growth was high in petroleum extract, ethyl acetate extract and hexane extract.

While microorganisms showed high resistance to aqueous extract of *Stevia*. *Streptococcus mutans* a common micro flora in mouth was susceptible to different extracts id SR, therefore it shows that SR leaves have capacity to prevent dental carries. Thus, *Stevia* leaves can be natural and potential antimicrobial agent against various microbes.

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8. Antimicrobial Activity of *Glycyrrhiza glabra* Linn (*Mulethi*)

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Abstract:

Glycyrrhiza glabra Linn (Gg) is a medicinal herb. In India, it is also known as Mulethi. It belongs to the family Fabaceae. Gg has been used for its anti-inflammatory, anti-ulcer,

anti-tumor, and other activities. Antimicrobial activity refers to the ability of a substance to inhibit the growth or kill microorganisms, such as bacteria, viruses, fungi, and protozoa. Dried roots, leaves, and rhizome extract of Gg showed significant anti-microbial activity by increasing the zone of inhibition in microbial strain.

Chalcones decrease the expression of bacterial genes, inhibit growth, and reduces the production of bacterial toxin. Methanol extract of Gg showed a maximum zone of inhibition in E. coli bacteria (33mm) hexane extract of Gg showed a minimum inhibition of zone in R. solani fungi (13mm).

The present review article discusses the anti-microbial activities of Gg that have been reported in scientific references.

Keywords:

chalcone, inhibition, E. coli, R. solani.

8.1 Introduction:

Glycyrrhiza glabra Linn (Gg) is an ancient herb; it belongs to a family of Leguminosae (1). It is native to Asia and Europe and is well-known for its sweet taste and distinct flavor (2). Dry roots and Rhizomes of Gg are widely used in clinical prescriptions.

This herb contains antiviral, anticancer, anti-ulcer, and anti-bacterial properties. The root contains a compound called glycyrrhizin, which is responsible for its characteristic sweet taste and numerous health benefits (3).

8.2 Taxonomy:

Kingdom: Plantae

Class: Dicotyledoneae

Order: Rosidae

Family: Leguminosae

Genus: Glycyrrhiza

Species: glabra Linn

Binomial name: *Glycyrrhiza glabra* Linn

(4)

8.3 Morphology:

Table 8.1: Morphology of Gg (5)

Plant part	Morphological characteristics
Root	Long, woody, and cylindrical, and can reach up to 1.5meters in length
Leaves	Leaflets are ovate to oblong in shape and are about 1-4cm long
Flowers	0.8-1.2cm long, pea-like flowers that are pale blue to violet in color

Figure 8.1: A-Roots, B-Leaves, C-Flower, D- Fruit

8.4 Vernacular Names:

Table 8.2: Vernacular Names of Gg (5)

Language	Names
Sanskrit	Yashti-madhu
Hindi	Jothi-madh, Mulethi
Kannada	Yastimadhuka, Atimadhura
Malayalam	Iratimadhuram
Tamil	Atimaduram
English	Licorice
Telugu	Yashtimadhukam

8.5 Phytochemistry of Gg:

Table 8.3: Phytochemical of Gg (6)

Compound	Chemical Constituent
Flavonoids	Liquiritin, Isoliquiritin, Liquiritigenin, Isoliquiritigenin Glabrolide, Licoflavonol
Isoflavones	Glabridin, Galbrene, Glabrone, Formononetin, Glyzarin, Licoisoflavones A and B
Saponins	Glycyrrhizin, Glycyrrhizic acid, Liquiritic acid Glycyrretol, Licorice acid

8.6 Pharmacological Activity of Gg:

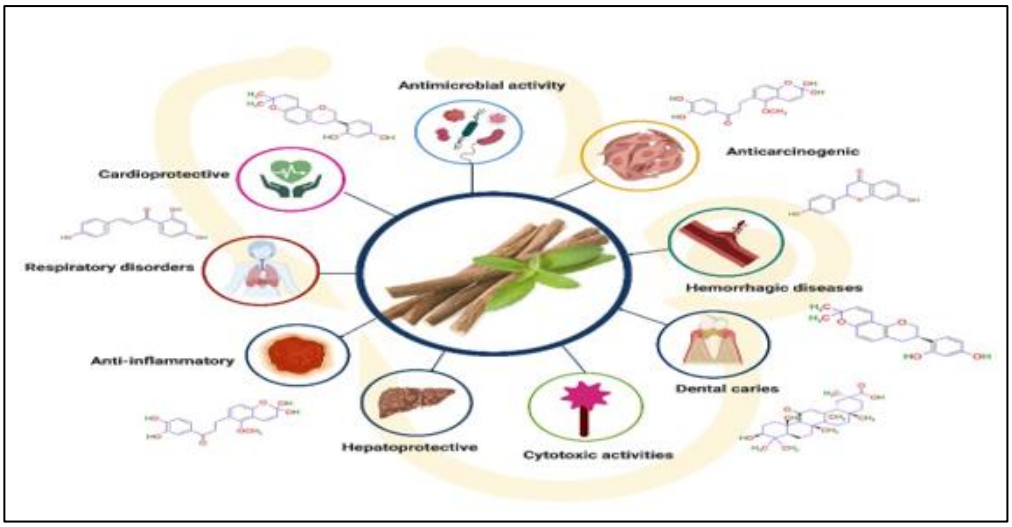

Figure 8.2: Pharmacologicl activity of Gg (6)

8.7 Antimicrobial Activity of Gg:

Rios *et al.,* in 2005 studied antimicrobial activity on *E.coli, S.aureus, A.niger, R.solani, A.flavor* by using different extract of Gg such as methanol, hexane, chloroform. Methanolic extract of Gg showed highest inhibition of zone 33 mm in *E.coli,* and *B.subtilis* 27.5 mm,butanol extract highest inhibition of zone *in S.aureus* 23.5 mm, *Aspergillus niger* 21.5 mm, *R.solani* 18 mm (7). Biondi *et al.,* in 2005 studied antimicrobial activity of Gg on bacterial strains. Methanolic extracts of Gg showed 16.3mm zone of inhibition and Acetonic extract showed 16 mm zone of inhibition in *E. coli.* bacteria. Methanolic extract showed a 15.3 mm zone of inhibition and Acetonic extract of showed a 15 mm zone of inhibition in *V.cholera* (8). Fan et al., in 2007 studied the anti-microbial activity of Gg on various micro-organisms strains such *as E.coli, B.subtilis, S. aureus, and P.aeurginosa.* Various extracts of Gg were used as a treatment to the microbial culture, and the zone of inhibition was measured. Tetracyclin extract showed highest inhibition zone on *E.coli, B.subtilis* whereas

Chloroform extracts showed least zone of inhibition (9). Runyoro et al., in 2006 studied the anti-microbial activity of Gg *on Klebsiella pneumonia* species. Ethanolic extracts (100 mg/ml) of Gg was used as a treatment on bacterial culture, and zone of inhibit were measured. It showed 17 mm inhibition of zone on comparison with standard Ciprofloxacin (30µg/ml) showed 22 mm inhibition of zone (10).

Table 8.4: Antimicrobialactivity of Gg [ME-Methanolic Extract, BE- Butanolic Extract, HE- Hexane Extract, CE-Chloroform Extract TE-Tetracyclin Extract, AE-Acetone Extract, SE-Streptomycin Extract]

Sr. No	Model	Treatment	Result (Zone of Inhibition) in mm	References
1	*E. coli*	Rifampcin [10mg/ml] ME [10mg/ml] CE [10mg/ml] BE [10mg/ml] HE [10mg/ml]	34.2 33 13 17 13.5	[7]
		ME [1.0g] AE [1.0g]	18.3 15.3	
		EE [1.2mg/ml] AE [1.2mg/ml] CHE [1.2mg/ml] TE [5mg/ml] STE [7.5mg/ml]	16 15 11 36 22	[8]
2	*B.subtilis*	Rifampcin [10mg/ml] ME [10mg/ml] CE [10mg/ml] BE [10mg/ml] HE [10mg/ml]	37 27.5 19 27.4 18.5	[7]
		EE [1.2mg/ml] AE [1.2mg/ml] CHE [1.2mg/ml] TE [5mg/ml] STE [7.5mg/ml]	19 22 16 34 22	[8]

Sr. No	Model	Treatment	Result (Zone of Inhibition) in mm	References
		ME [1.0g] AE [1.0g]	18.6 14.3	[9]
3	*S. aureus*	Rifampcin [10mg/ml] ME [10mg/ml] CE [10mg/ml] BE [10mg/ml] HE [10mg/ml]	29.4 19 15.5 23.5 18.5	[7]
		ME [1.0g] AE [1.0g]	17.6 15	[9]
		EE [1.2mg/ml] AE [1.2mg/ml] CHE [1.2mg/ml] TE [5mg/ml] STE [7.5mg/ml]	22 32 18 25 26	[8]
		Ciprofloxacin [30μg/ml] EE [100mg/ml] EE [100mg/ml]	25 15 13	[10]
4	*A.niger*	Fluconazole [10mg/ml] ME [10mg/ml] CE [10mg/ml] BE [10mg/ml] HE [10mg/ml]	17.5 16.5 13 21.5 14.5	[7]
5	*R. solani*	Fluconazole [10mg/ml] ME [10mg/ml] CE [10mg/ml] BE [10mg/ml] HE [10mg/ml]	23 16 12.5 18 13	[7]
6	*A.flavus*	Fluconazole [10mg/ml] ME [10mg/ml]	31.5 17.5 30.5	[7]

Sr. No	Model	Treatment	Result (Zone of Inhibition) in mm	References
		CE [10mg/ml] BE [10mg/ml] HE [10mg/ml]	19 14	
7	*S.typhi*	ME [1.0g] AE [1.0g]	16.3 16	[9]
8	*V.cholera*	ME [1.0g] AE [1.0g]	15.3 15	[9]
9	*B.cereus*	ME [1.0g] AE [1.0g]	17.6 16.3	[9]
10	*Klebsiella pneumonia*	Ciprofloxacin [30μg/ml] EE [100mg/ml]	22 17	[10]
11	*P.aeurginosa*	EE [1.2mg/ml] AE [1.2mg/ml] CHE [1.2mg/ml] TE[5mg/ml] STE[7.5mg/ml]	14 22 14 32 31	[8]

8.8 Conclusion:

Gg is a well-known medicinal herb from the family Fabaceae. The present review summarizes the antimicrobial activity of Gg. Methanol extract of Gg showed a maximum inhibition of zone in *E. coli* bacteria (33mm). Hexane extract showed a minimum zone of inhibitionn in *R.solani* fungi (13mm). Hence the Gg showed good antimicrobial activity.

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9. Development of Banana Dose from Foxtail millet (*Setaria italic*)

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Abstract:

Dose is a traditional South Indian breakfast dish, made out of rice and black gram dal. The banana dose is a type of dose made in the South Karnataka and Kerala region using a base as rice and banana. In this study rice is partially replaced by Foxtail millet (Setaria italica), it possesses health-promoting properties owing to its unique protein composition containing a high content of essential amino acids. The mature foxtail seeds mainly consist of proline-rich, alcohol-soluble proteins (prolamin) called setarins. Banana is rich in fibre, potassium and magnesium helps in digestion, boost bone health and build lean muscle. In this study, banana doses were prepared by partially replacing rice with Foxtail millet (FM). Six formulations (FMBD1, FMBD2, FMBD3, FMBD4, FMBD5 and FMBD6) containing

different proportions of FM (0%, 20%, 40%, 60%, 80%, 100%) along with rice and banana were used to prepare dose. These formulations were analyzed for sensory attributes. Further, standardization of fermentation time and analysis of proximate composition was also carried out. Banana dose prepared with 60% Foxtail millet fermented for 12 hrs had the highest sensory scores. The nutritional composition of the banana dose prepared from FM had high protein, fibre, phosphorus and less fat and carbohydrate content compared to the standard banana dose.

Keywords:

Foxtail millet (FM), fermentation, protein, acceptability, proximate.

9.1 Introduction:

Fermented foods are those whose production involves the action of micro-organisms or enzymes which cause desirable biochemical changes and significant modification to the food.

Lactic acid bacteria, moulds of *Aspergillus* spp., *Penicillium* spp. and of Mucorales, and yeasts, often of *Saccharomyces* spp. are most important (1, 2).

Dose is a traditional South Indian breakfast dish, made out of rice and black gram dal. The banana dose is a type of dose made in the South Karnataka and Kerala region using a base as rice and banana.

Millets contain more vitamins and minerals than rice, Foxtail millets (*Setaria italica*) are the world's second most cultivated millet, it possesses health-promoting properties owing to their unique protein composition containing a high content of essential amino acids.

The mature foxtail seeds mainly consist of proline-rich, alcohol-soluble proteins (prolamin) called setarins, comprising about 60% of the total protein, with less content of disulfide cross-linked proteins than with other cereal and millet. It is rich in Vitamin B12 which is essential for maintaining a healthy heart and smooth functioning of the nervous system.

A diet including FM may improve glycemic control and reduce insulin, cholesterol and fasting glucose in Type-2 diabetes patients (4, 5). Banana is rich in fibre, potassium and magnesium helps in digestion, boost bone health and build lean muscle. Potassium benefits the muscles as it helps maintain their proper working and prevents muscle spasms. Several antioxidants and flavonoids are found in bananas, most notably catechins. They have been linked to various health benefits, including a reduced risk of heart disease (6, 7, 8).

9.2 Objectives:

- To develop a millet-based fermented product and evaluate its organoleptic properties.
- To determine the optimum fermentation time on the developed product.
- To develop organoleptic acceptability of the developed product.

9.3 Materials and Methods:

9.3.1 Raw Materials:

- The present study was carried out in the Department of Food Science and Nutrition, Yuvaraja's College (Autonomous), Mysuru.
- The raw materials viz., rice, Foxtail millet, banana and salt were procured from the local market of Mysuru, Karnataka, India.
- **Method of preparation:** Banana dose was prepared by soaking Foxtail millet and rice for 4 hours. Rinse the soaked FM and rice then drain the water. Grind the FM and rice along with the banana make the batter into thick consistency add salt mix well and leave it for 12 hours for fermentation. On a medium flame, heat the tawa and cook both sides by adding ghee.
- **Optimization of batter fermentation:** Foxtail millet and rice were mixed in different proportions (Standard, 20%, 40%, 60%, 80% & 100%) and were allowed to soak for 4 hrs. After soaking they were ground along with banana and allowed to ferment for 6, 12, 18, 24 hours respectively at room temperature.
- **Determination of leavening index and pH**: The change in volume & pH, were determined for all the compositions at room temperature by varying the time of fermentation. The pH was determined by the use of a pH paper by dipping them in the batter and the leavening index was determined by pouring the batter into a 250 ml measuring cylinder. The volume was noted in 6, 12, 18 & 24 hours.
- **Sensory Analysis of Prepared Foxtail Millet banana dose:** Sensory evaluation was conducted to determine the acceptability of various attributes such as appearance, taste, texture, colour, flavour and overall acceptability. The product was evaluated by taking the average score of the 30 semi-trained panel=lists by using a 9-point hedonic scale.
- **Nutritional analysis of Prepared Foxtail Millet banana dose:** Standard A.O.A.C. (1980) method was used to determine the Nutritional composition of selected variation (FMBD4) of Foxtail millet banana dose and control (9). The moisture content was estimated by using a hot air oven at 98 to 100˚ C, Protein content was estimated by determining total nitrogen content using the standard Micro - Kjeldhal method (10, 11), ash % was estimated by high-temperature incineration using muffle furnace (12) and fat content was estimated by the Soxhlet method (13, 14). The crude fibre content was estimated by a crude fibre analyzer. The carbohydrate content was obtained by subtracting from 100 with the sum of values of moisture, protein, fat and ash content per 100 g of the sample. Minerals like Calcium, iron and phosphorous were analyzed using inductively coupled plasma mass spectrometry (ICPMS). These methods give good precision and accuracy (15).
- **Statistical analysis:** Each sample was analyzed in triplicates. The data obtained were analyzed statistically using standard methods given by Snedecor and Cochran (16) and by Duncan's multiple range test with the $p \leq 0.05$ consider to be significant (17).

9.4 Formulations of Foxtail Banana Dose:

Table 9.1: Formulations of Foxtail banana dose

Ingredients	0%	20%	40%	60%	80%	100%
Rice (g)	100	80	60	40	20	-
Foxtail millet (g)	-	20	40	60	80	100
Banana (g)	100	100	100	100	100	100
Salt (g)	3	3	3	3	3	3

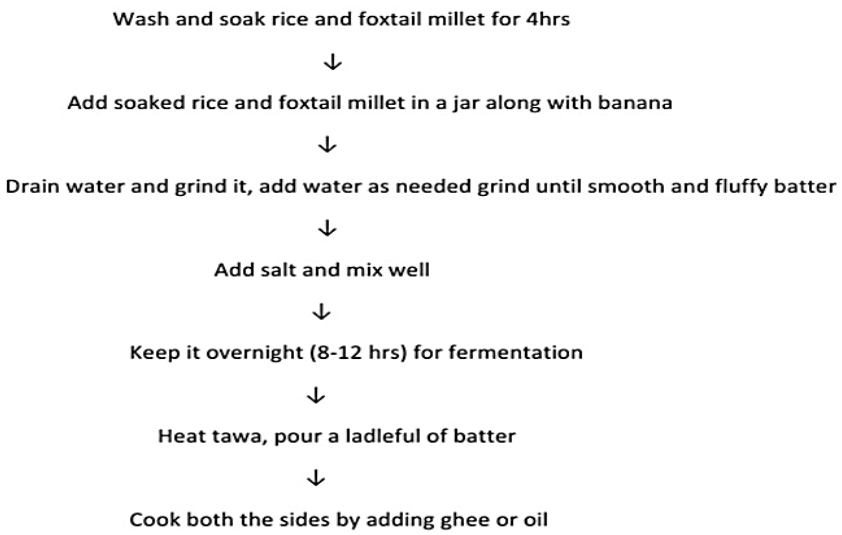

Figure 9.1: Flow Chart of Preparation of Foxtail Banana Dose

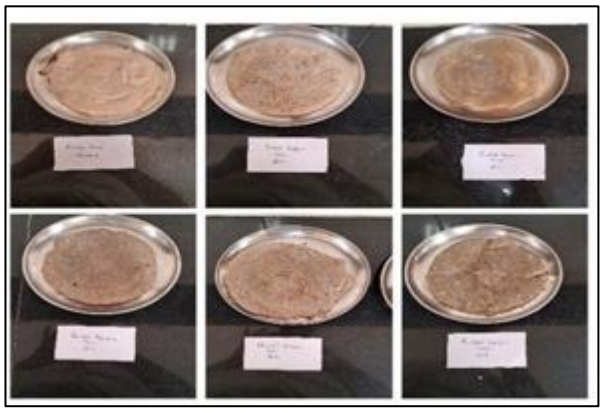

Figure 9.2: Formulations of Foxtail banana dose

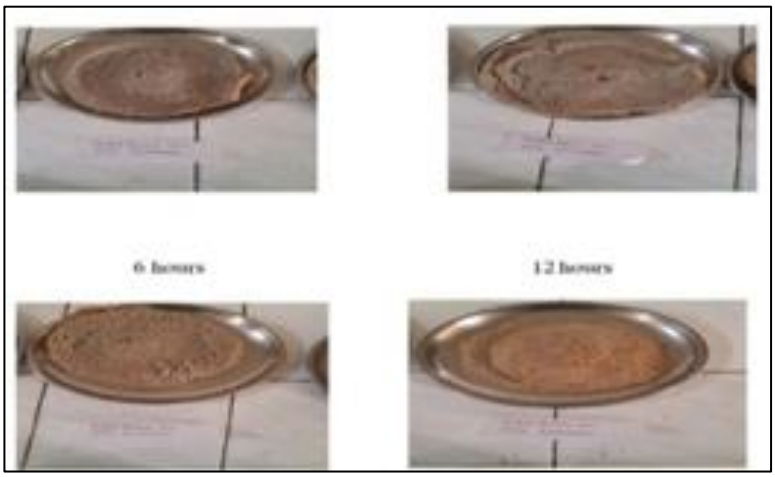

Figure 9.3: Foxtail banana dose Prepared by varying Banana dose

9.5 Results and Discussion:

9.5.1 Organoleptic evaluation of Foxtail Millet Banana dose:

The study considered preparing a millet-based banana dose by partially replacing rice with Foxtail millet. The data pertaining to the effect of the incorporation of various levels of FMBD (20, 40, 60, 80 and 100%) on sensory attributes of FMBD are shown in Table 9.2. The results obtained for all sensory attributes that FMBD2, FMBD3 and FMBD4, were similar to the control, whereas FMBD5 and FMBD6 showed decreased scores and were less acceptable compared to other variations.

The acceptable FMBD4 variation was incubated for different fermentation times to study the optimum fermentation time of Foxtail millet incorporated banana dose. Data pertaining to sensory evaluation of Foxtail banana dose with respect to appearance, colour, taste, texture, flavour and overall acceptability were carried out. Accordingly, the results obtained are depicted in Table 9.2.

Table 9.2: Organoleptic evaluation of Foxtail Millet banana dose

Parameters	Appearance	Colour	Texture	Taste	Flavour	Overall acceptability
FMBD1	9±0.0	9±0.0	9±0.0	8.9±0.53	8.9±0.47	8.9±0.53
FMBD2 (20%)	8.9±0.3	8.85±0.357	8.8±0.509	8.85±0.357	8.9±0.3	8.85±0.37
FMBD3 (40%)	8.5±0.43	8.3±0.45	8.42±0.57	8.35±0.57	8.64±0.4	8.5±0.32

Parameters	Appearance	Colour	Texture	Taste	Flavour	Overall acceptability
FMBD4 (60%)	8.5±0.57	8.4±0.54	8.6±0.57	8.5±0.4	8.75±0.365	8.7±0.42
FMBD5 (80%)	8.1±0.32	8.2±0.57	8.1±0.35	8.15±0.25	8.21±0.37	8.24±0.53
FMBD6 (100%)	7.75±0.4	7.9±0.4	7.7±0.53	7.87±0.36	7.89±0.57	7.9±0.23

Values are mean SD, p ≤0.05 (Holm Sidak method), n=30

9.5.2 Sensory Evaluation Table of Foxtail Millet Banana Dose Prepared by Varying Fermentation Time:

The data pertaining to the effect of incubation of different fermentation times of FMBD (6hrs, 12hrs, 18hrs and 24hrs). The sensory attributes of FMBD by varying fermentation times FMBD6, FMBD12, FMBD18 and FMBD24 are shown in Table 9.3. The variation FMBD12 was more acceptable in terms of sensory attributes.

Table 9.3: Foxtail Millet banana dose prepared by varying fermentation time

Parameters	Appearance	Colour	Texture	Taste	Flavour	Overall acceptability
FMBD6 (6hr)	6.888±0.314*	7.277±0.558	6.555±1.012*	6.833±0.7637*	6.444±0.955*	6.944±0.704*
FMBD12 (12hr)	7.352± 0.477	7.352± 0.966	7.652± 0.477	7.917± 0.4705	7.941±0.539	7.87±0.605
FMBD18 (18hr)	7.288± 0.590	7.244± 0.598	7.433± 0.666	7.744± 0.911	7.666± 1.05	7.688±0.737
FMBD24 (24hr)	7.152± 0.477	7.052± 0.966	7.176± 0.705	6.705± 0.665*	6.882± 0.675*	7.05±0.539*

[Values are mean SD, p ≤0.05 (Holm Sidak method), n=30]

9.5.3 pH of FMBD Batter:

pH value of FMBD batter at different fermentation periods ranged from 5 to 6 (6, 12, 18, 24 hrs). The initial pH was 6.0, as fermentation occurred the pH was 5.0 throughout the process even after varying the fermentation time.

There is an increase in acidity level, i.e., a decrease in the pH value. This is mainly due to the development of Lactic acid bacteria which produces lactic acid which lowers the pH, and the production of carbon dioxide, which leavens the batter.

Table 9.4: pH of FMBD Batter

Variations	Initial pH	pH after 6 hrs	pH after 12 hrs	pH after 18 hrs	pH after 24 hrs
Standard (FMBD1)	6	5	5	5	5
FMBD2 (20%)	6	6	6	5	5
FMBD3 (40%)	6	6	6	5	5
FMBD4 (60%)	6	6	5	5	5
FMBD5 (80%)	6	6	5	5	5
FMBD6 (100%)	6	6	5	5	5

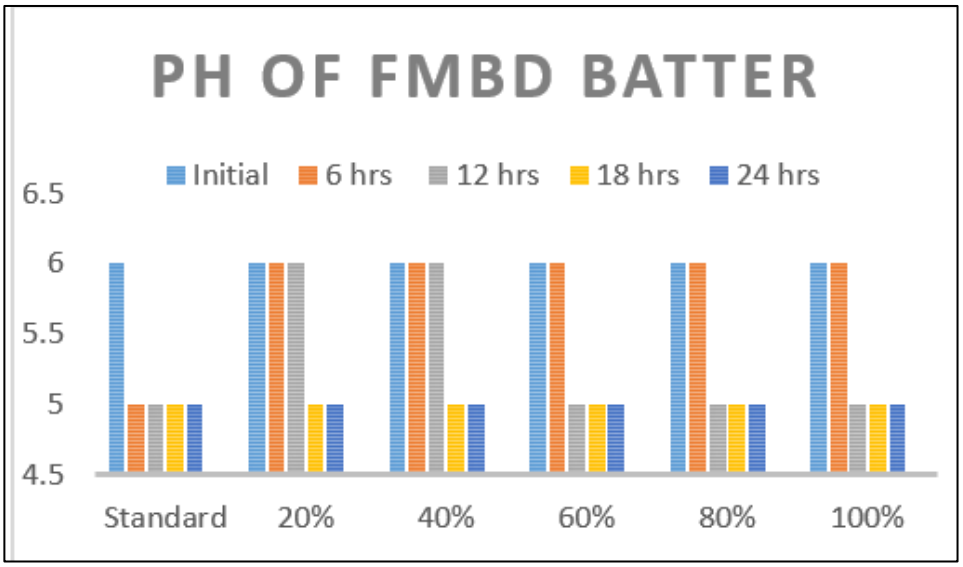

Figure 9.4: pH of FMBD Batter

9.5.4 Volume of FMBD Batter:

The initial volume of the batter was 25 ml for all the variations and increased gradually till 24 hrs. At 24 hours the volume of batter was reduced in 20%, 80% and 100%.

This increased volume of FMBD batter is due to an increase in lactic acid bacteria in the batter during fermentation and entrapment of air.

Table 9.5: Determination of the volume of FMBD Batter

Variations	Initial volume (ml)	volume after 6 hrs (ml)	Volume after 12 hrs (ml)	Volume after 18 hrs (ml)	Volume after 24 hrs (ml)
Standard (FMBD1)	25	25	30	35	40
FMBD2 (20%)	25	25	30	35	35
FMBD3 (40%)	25	25	30	35	40
FMBD4 (60%)	25	25	30	35	40
FMBD5 (80%)	25	25	30	35	35
FMBD6 (100%)	25	25	30	35	35

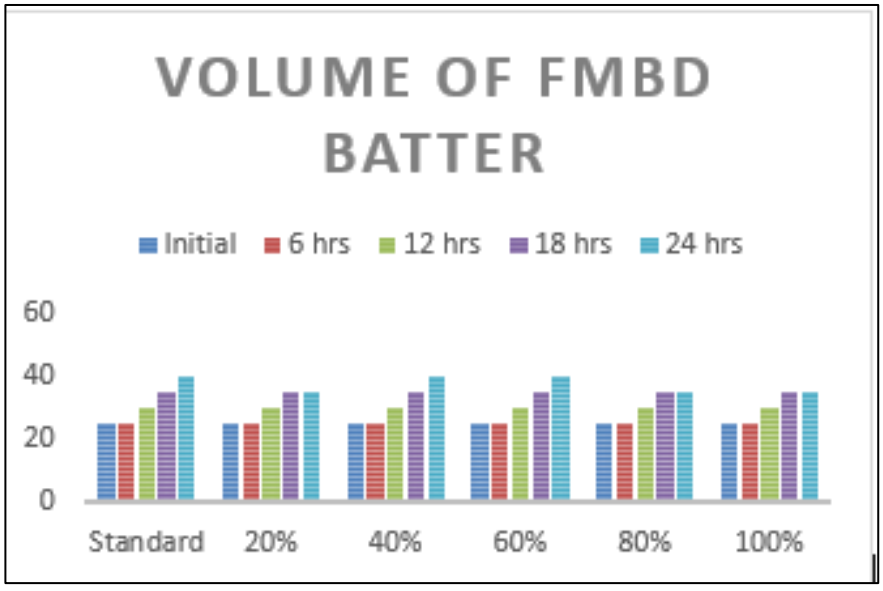

Figure 9.5: Volume of FMBD Batter

9.5.5 Nutritional Composition of the Accepted Formulation:

The proximate composition of the acceptable FMBD4 and that of control were analyzed and the results of the same are shown in Table 9.6. The moisture content of all the variations of FMBD was similar. The values of protein, fibre and calcium content values were higher in FMBD4 than in the control, whereas carbohydrate was less. However, ash, iron and phosphorous content were increased in the Foxtail banana dose.

Table 9.6: Nutritional Composition of control and FMBD4 dose

Nutrients/100 g	Control (FMBD1)	FMBD4 (60%)
Moisture (%)	60.12 ± 2.12	63.3± 1.81
Carbohydrates (g)	70.04± 1.10	60.2± 1.6
Protein (%)	6.84± 0.71	9.90 ± 0.83*
Fat (g)	0.63± 0.15	0.52± 0.28
Crude Fibre (%)	0.67± 0.13	2.03± 0.3*
Ash (%)	1.35± 0.11	2.05± 0.1
Energy (Kcal)	219± 3.11	299.6± 3.05*
Iron (mg)	0.26± 0.11	1.94± 0.32
Calcium (mg)	11.07± 1.09	23.6± 1.01*
Phosphorus (mg)	174.0± 1.19	210.0± 2.02*

9.6 Conclusion:

Fermented products get easily digested and by incorporation of banana and Foxtail millet, it more easily gets digested and is low in fat and high protein content. The incorporation of 60% Foxtail millet (FMBD4) had the highest acceptability in terms of sensory scores. Whereas, FMBD5 (80%) and FMBD6 (100%) had the least acceptability. As a result of poor fermentation, FMBD6 (6hr) had the lowest sensory scores and FMBD24 (24hr) had the lowest sensory scores due to hyper fermentation. Proximate analysis of nutrients in selected variations of dose had significantly increased levels of dietary fibre, iron and low in fat and carbohydrate content when compared to standard. The optimum fermentation time for foxtail millet banana dose was 12hr.

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10. Effect of Fermentation on Sensory Attributes of Idli Prepared by Incorporation of Little Millet (*Panicum Sumatrense*)

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Abstract:

Idli is a fermented food of India which is prepared by steaming a fermented black gram dal and rice batter. It makes an important contribution in the diet as a source of calories, proteins, vitamins especially B-complex vitamins. Lactic acid bacteria are responsible for the fermentation which are present in the idli batter. Cereals and legumes are the main ingredients in the idli batter but it can be replaced with millets. Millets are rich in minerals, tannins, flavonoids, and antioxidants. Millets are also rich in dietary filers, magnesium and niacin and it is low in glycemic index.

Little millet is rich in magnesium which helps improve heart health. It is also rich in niacin which helps lower cholesterol. Little millet contains phosphorous which is great for weight loss. It contains probiotics which improves the gut health. This study was conducted to develop and evaluate idli of six different composition L1, L2, L3, L4, L5 and L6. Standardization trials indicated that incorporation of little millet at [0%, 20%, 40%, 60%, 80%, 100%] were incorporated to the standard recipe. These incorporated formulations were analysed for sensory attributes [n=20]. The sensory score is highest for L3. Idli prepared with 40% little millet and fermented for 12 hrs had the the highest scores in terms of sensory attributes. Little millet rice idlis is nutritionally superior than the control idlis as it is rich in fibre and magnesium, niacin and antioxidants.

Keywords:

little millet, fermented, lactic acid bacteria, cereals and legumes.

10.1 Introduction:

Millets are one among the cereals apart from the rice, wheat, maize and barley. Cereals are the most common diet in most of the developing nations. Millets has many properties one among them is being gluten free, which helps in treating celiac disease and gluten allergy [1].

Millets has high number of proteins, dietary fibre, carbohydrates, phytochemical and micronutrients. In the present era of food scarcity, there exists a need of diversity to use the millets by developing millet products. Millet grains are also known as 'famine reserves' because the grains can be stored upto two or more years [2].

Little millet (Panicum *smartens*) is one of the minor millets it is known as saamai or kutki contains high energy value and are nutritious compared to other cereals.

Little millet is a short duration millet which withstands both drought and water logging. Little millet has another name known as cool food because of the cooling impact it has on the body when consumed during the summer season [3].

idli is a popular fermented food of India and Sri Lanka. It is cereal legume-based product. Idli is steam cooked from the fermented batter of black gram dal and rice [4].

Idli provides balanced amount of carbohydrate, proteins and B-vitamins. The standard idli batter preparation consists of three major steps - soaking of rice and black gram dal for 6 hrs, wet grinding and fermentation for 12hrs [5].

Fermentation is an anaerobic process in which energy can be released from glucose even if the oxygen is not available**.** Microorganisms involved in fermentation are bacteria, yeast and molds more specifically lactic acid bacteria are involved in fermentation process. [6]**.**

Fermenting the batter improves the nutritional and organoleptic properties of the idli [5].

Figure 10.1: Little Millet (Panicum *Sumatrense*)

10.2 Objective:

- To develop idli from little millet.
- To evaluate organoleptic acceptability of developed idli.
- To study the effect of fermentation time.

10.3 Materials and Methods:

- **Raw materials:** The present study was carried out in the department of food science and nutrition, Yuvaraja's college, University of Mysore, Mysore. The raw materials such as little millet, parboiled rice, black gram dal and salt were produced from the local market of Mysuru.
- **Method of preparation:** idli was prepared by soaking different ration of parboiled rice, black gram dal and little millet for 6 hrs. The soaked grains are grinder into coarse paste and water was added to adjust the consistency of the batter. The batter was fermented for 12 hrs in a warm place. After the batter is fermented add salt and stir the batter well. Steam the batter in idli cooker for 20 mins. Idli is ready to serve.
- **Sensory analysis of idli:** Sensory evaluation of idli was done for the sensory attributes such as appearance, color, texture, taste, flavor and overall acceptability to determine the acceptability of idlis. The product was evaluated by 30 semi-trained panelists using 9-point hedonic scale method.
- **Optimization of batter fermentation:** The batter of selected variation (P4) was allowed to ferment for different time periods (6 h, 12 h, 18 h and 24 h).
- **pH and Volume:** For the different fermentation time of selected variation (P4) batter, the batter properties viz., pH and volume were studied. pH was recorded initially and at the end of fermentation using pH paper. Initial batter volume and volume raised after fermentation was measured using measuring cylinder.
- **Nutritional analysis of prepared papad :** Standard AOAC (1980) method was used to determine the nutritional composition of selected variation (L3) of little millet idli and control. The moisture content was estimated by using hot air oven at 98 to 100 °C, protein content was estimated by determining total nitrogen content using standard Micro- kjeldhal method, ash % were estimated by high temperature incineration using

muffle furnace [7,8] and fat content was estimated by the soxhlet method. The crude fibre content was estimated by crude fibre analyser. The carbohydrate content was obtained by subtracting from 100 with the sum of values of moisture, protein, fat and ash content per 100g of the sample. Minerals like calcium, iron and phosphorous were analysed using inductively coupled plasma mass spectrometry (ICPMS). These methods give a good precision and accuracy [7].

- **statistical analysis:** Each sample was analysed in triplicates. The data obtained was analysed statistically using standard methods given by Snedecor - and Cochran [9] and by Duncan's multiple range test with the $p \leq 0.05$ consider to be significant [10].

10.4 Formulation of the Product:

wash and soak little millet, parboiled rice and black gram dhal for 6 hrs

↓

Grind them coarsely, add water to adjust the consistency

↓

Allow the batter to ferment for 12 hrs in warm place

↓

Add salt and mix the batter well

↓

Steam the batter in idli cooker for 20 mins

↓

Idli is ready to serve

Figure 10.2: Flow Chart for the Preparation of Little Millet Idli

Table 10.1: formulation of the product (ingredients g/100g)

Ingredients	L1 Standard	L2 (20%)	L3 (40%)	L4 (60%)	L5 (80%)	L6 (100%)
Parboiled Rice	100	80	60	40	20	0
Black gram dhal (g)	35	35	35	35	35	35
Little millet (g)	0	20	40	60	80	100
Salt (g)	2	2	2	2	2	2

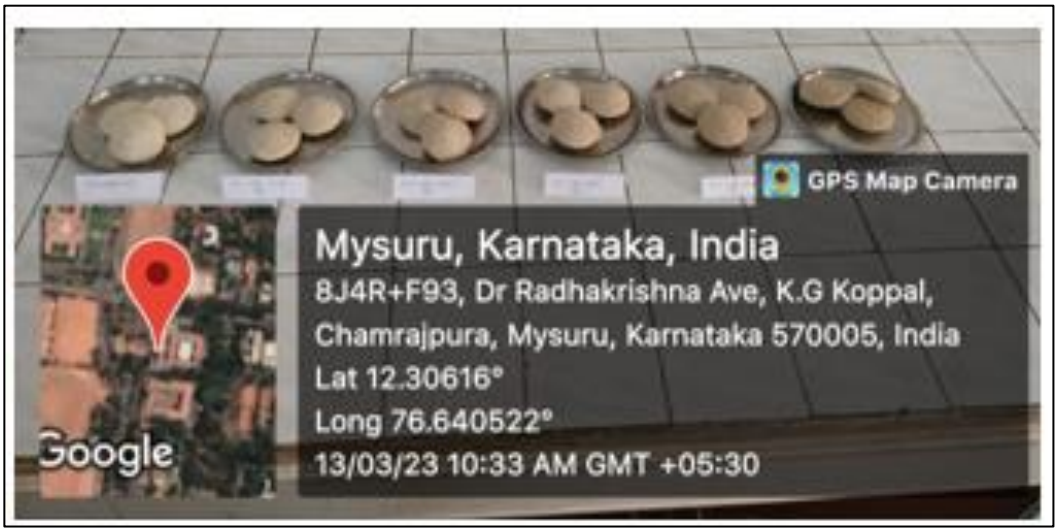

Figure 10.3: Standard Idli and Idli's Prepared by Incorporation of Little Millet

Figure 10.4: Little Millet Incorporated Idlis Prepared by Varying Fermentation Time

10.5 Results and Discussion:

10.5.1 Sensory Evaluation of Little Millet Idli:

The study was undertaken to prepare millet based idli by partially replacing rice with little millet grains. The data pertaining to the effect of incorporation of various levels of little millet grains (20%, 40%, 60%, 80%, 100%).

The sensory attributes of idli and the results are shown in Table 10.2. The scores obtained for all sensory attributes for LM2, LM3, LM4, LM5 and LM6. The variation LM3 were more acceptable in terms of sensory attributes.

Table 10. 2: sensory evaluation of different variation of idli developed from little millet with partial replacement of rice. Values are mean ± SD, $p \leq 0.005$ (Holm Sidak method), n = 30.

Variation	Appearan	Colour	Texture	Taste	Flavour	Overall
LM1 (standard)	9 ±0.0	9±0.0	9±0.0	9±0.0	9±0.0	9±0.0
LM2 (20%)	8.06±0.25	8.06±0.2	7.86±0.74*	7.86±0.7	7.86±0.74	7.9±0.45
LM3 (40%)	8.09±0.4	8.06±0.2	8±0.3	8.0±0.3	7.9±0.45*	8.0±0.25
LM4 (60%)	7.73±0.45	7.33±0.4	7.2±0.67*	7.26±0.7	7.33±0.61	7.33±0.48
LM5 (80%)	6.9±0.45	6.86±0.5	6.66±0.6	6.53±0.7	6.40±0.82*	6.60±0.6
LM6 (100%)	6.53±0.74	6.33±0.72	6.21±0.67	6.06±0.79	6.0±0.84*	6.13±0.83

10.5.2 Optimization of Fermentation Time:

The acceptable LM3 variation was incubated for differentfermentation time to study the optimum fermentation time of little millet incorporated idli. The data pertaining to the effect of incubation of different fermentation time of little millet grains (6hrs, 12hrs, 18hrs, 24hrs). The sensory attributes of idli and the results are shown in Table 10.3. The scores obtained for all sensory attributes of L1, L2, L3 and L4. The variation L2 was more acceptable in terms of sensory attributes.

Table 10.3: sensory evaluation of acceptable idli developed from little millet with partial replacement of rice incubated at different fermentation time. Values are mean ± SD, $p \leq 0.005$ (Holm Sidak method), n = 30.

Variation	(L1) 6hrs	(L2) 12 hrs	(L3) 18 hrs	(L4) 24 hrs
Appearance	7.91±0.23	8.01±0.7	7.71±0.23	7.53±0.61*
Colour	7.93±0.25	8.0±0.25	7.67±0.35	7.41±0.28*
Texture	7.72±0.45	7.82±0.45	7.53±0.33	7.32±0.45*
Taste	7.67±0.35	7.73±0.33	7.53±0.33	7.20±0.53*
Flavour	7.77±0.72	7.81±0.28	7.41±0.28	7.13±0.70*
Overall acceptability	7.86±0.35	7.99±0.45	7.73±0.45	7.33±0.61*

10.5.3 pH Value:

The pH of the little millet idli batter was measured using the ph paper. The figure 10.4 shows the ph of the idli batter sample. The initial pH of the batter was 6 and it was same for all the variations. The pH was decreased after fermentation, for 6, 12, 18, 24 hours of fermentation pH was decreased from 6 to 5 accordingly. Black gram dal soaked in water has a higher concentration of soluble nutrients which support the growth of lactic acid bacteria. The role of the lactic acid bacteria is to reduce pH of the batter to an optimum level for the yeast activity in the idli batter.

Table 10.4: pH value of idli batter

Variations	Initial pH	pH after 6h	pH after 12h	pH after 18h	pH after 24h
Control	6	5	5	5	5
LM2 (20%)	6	5	5	5	5
LM3 (40%)	6	5	5	5	5
LM4 (60%)	6	5	5	5	5
LM5 (80%)	6	5	5	5	5
LM6 (100%)	6	5	5	5	5

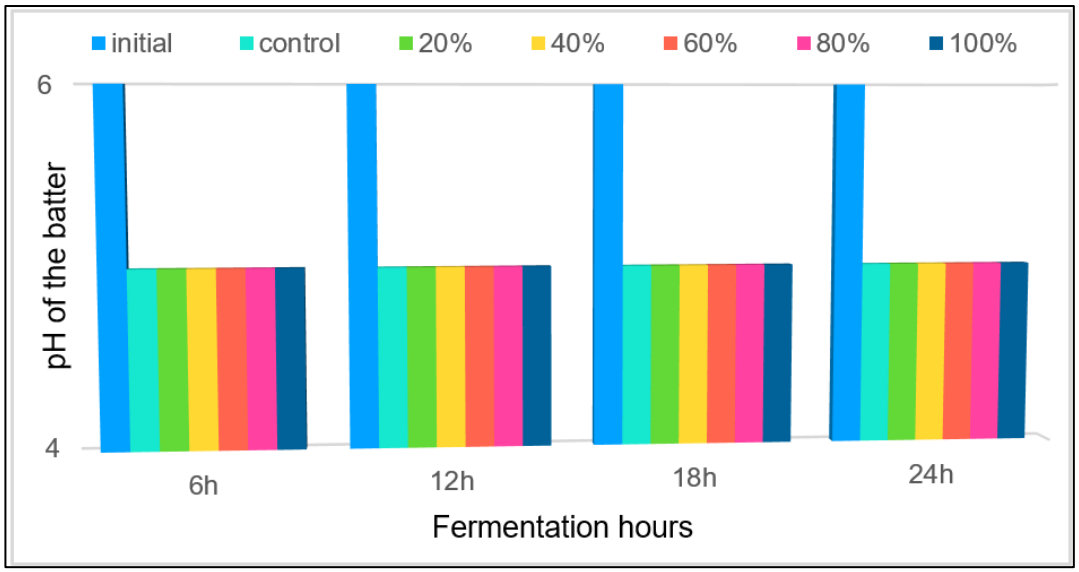

Figure 10.5: Determination of pH of LM Incorporated idli Batter

10.5.4 Volume:

The volume of the little millet idli batter was measured before and after optimum fermentation of the batter. The batter is fermented in a warm place (30 to 32°C) for 6, 12, 18 and 24 hrs, during the fermentation time, heat allows the growth of bacteria like yeasts along with free availability of oxygen and sugar decomposes into water and carbon-dioxide. Water dissolves but the carbon dioxide forms bubbles which increases the consistency of the batter and swells it. The initial volume of the batter before fermentation was 65ml and the volume of the batter after fermentation for all the variations were increased gradually till 18 hours. At 24 hours the volume was reduced. The lactic acid bacterium demonstrates the leavening action in the fermented idli batter and it also incorporates air that is carbon dioxide into the batter. The two species *L. mesenteroides* and *S. faecalis* mainly present the fermented batter. *L. mesenteroides* is essential for leavening of the batter along with *S. faecalis* is responsible for acid production. Both the functions are essential for producing a satisfactory idli.

Table 10.5: Volume of idli Batter

Variations	Initial volume (ml)	Volume after 6h (ml)	Volume after 12h (ml)	Volume after 18h (ml)	Volume after 24h (ml)
Control	65	75	75	77	72
LM2 (20%)	65	69	73	79	74
LM3 (40%)	65	71	75	82	79
LM4 (60%)	65	74	77	86	81
LM5 (80%)	65	80	84	90	84
LM5 (100%)	65	83	89	92	87

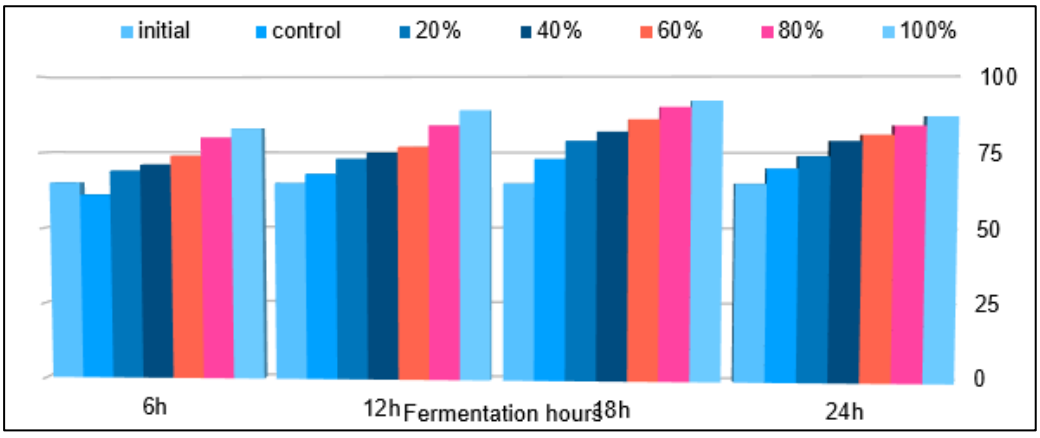

Figure 10.6: Volume of Idli Batter

10.5.5 Proximate Composition of Prepared Idli:

The proximate composition of acceptable little millet idli (LM3) and that of control were analysed and the results of the same are shown in Table 10.6. The moisture content of all the variations of idli was similar. The values of protein and dietary fibre content was higher in LM3 than that of control, whereas carbohydrate was less. However, ash, iron and phosphorous content were increased in little millet idli.

Table 10.6: proximate composition of selected variation [LMI (40%)] of idli developed from little millet with replacement of rice. Values are mean ± SD, $p \leq 0.005$ (Holm sidak), $n = 3$

Nutrients / 100g	Standard	LMI (40%)
Moisture (%)	24.03±0.15	21.08±0.25*
Carbohydrate (g)	60.90±0.95	59.18±0.45
Protein (g)	11.81±1.10	16.77±0.48*
Fat (g)	0.84±0.35	1.76±0.35*
Crude Fiber (g)	0.83±0.03	0.16±0.05*
Ash (g)	1.59±0.02	1.70±0.04*
Energy (kcal)	328±0.19	395±0.15*
Iron (mg)	1.96±0.12	6.2±0.04*
Phosphorous (mg)	95.37±0.12	128.2±0.53*

10.6 Conclusion:

Idli is a type of savory rice cake, originating from South India, popular as a breakfast food in Southern India and in Sri Lanka. The cakes are made by steaming the batter consisting of fermented black lentils and rice. Here we have attempted to develop idli using little millet. It is rich in proteins, calories and B-vitamins. Nutrients present in black gram dhal effects the growth of microorganisms during fermentation.

The role of the lactic acid bacteria is to reduce the pH of the batter to an optimum level for the yeast activity in the idli batter. The two species *L. mesenteroides* and *S. faecalis* mainly present the fermented batter of idli. *L. mesenteroides* is essential for leavening of the batter along with *S. faecalis* is responsible for acid production. Standardisation of fermentation time for selected variation resulted in more acceptability in terms of sensory attributes with maximum scores for L2 (12h).

Little millet idli of 40% (LM3) had highest acceptability in terms of sensory scores where as other variations had least acceptability.

Little millet idli had increased level of protein, dietary fibre, iron and phosphorous. The carbohydrate content was low compared standard making it low in glycemic index. The optimum fermentation time for little millet incorporated idli was found to be 12h and acceptable up to 40%.

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11. Standardization of Fermentation Time and Its Effect on Sensorial Attributes of Bhatura from Barnyard Millet (*Echinochloa frumentacea*)

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Abstract:

Fermentation has been a major way of preserving foods. Microbial growth causes chemical and textural changes to form a product that can be stored for extended periods. Bhatura is a North Indian leavened fried bread made with a leavened dough of maida flour, curd, semolina, salt, sugar, and a leavening agent (baking soda, baking powder). Barnyard millet has become one of the most important minor millet crops in Asia, showing a firm upsurge in world production. It is a good source of protein, carbohydrates, and fiber, and most notably, contains more micronutrients (iron and zinc) than other major cereals. It is believed that the consumption of barnyard millet can possess various health benefits against diabetes (as the flour is gluten-free), cardiovascular diseases, obesity, skin problems, cancer, and celiac disease. In this study, Bhatura was prepared in six different variations like B1, B2, B3, B4, B5, and B6 with partially replacing maida flour with barnyard millet. The different incorporation of barnyard millet proportion was (0%, 20%, 40%, 60%, 80%, 100%). These different formulations of bhatura dough were fermented for different timings like 1, 2, 3, 4 h and standardized fermentation time was analysed and was analysed for their sensorial attributes by hedonic scale method (n=15). The sensory scores were highest for Bhatura which had 40% barnyard (B3) and was fermented for 3 h. The proximate nutritional values in this variation were high in iron, protein, fat, and fiber compared to the standard bhatura. This study helps in understanding the relation between fermentation time and sensorial properties of the Bhatura which also is gluten-free and have low glycaemic index.

Keywords:

Fermentation, Leavening, Lactobacillus, celiac disease, Barnyard millet.

11.1 Introduction:

Bhatura or puffed fried bread is a popular traditional sourdough bread commonly served as a midday meal or as breakfast in northern and eastern parts of the Indian subcontinent (1). It is a large puffy fried bread made out of maida (Bleached and refined wheat flour). It is often eaten in combination with chole (spicy chickpeas curry) mainly eaten in North India and along with chole seems to be originated in Punjab. It is a snack food sold by vendors on the street and a very common item on the menu of a party (2). A typical recipe in the preparation of bhatura includes white flour, curd, and oil. Once kneaded, the dough is left to rise, and then small balls are plucked out of it either hand rolled or flattened using a rolling pin, Then the bread is deep fried until they puff up into a light brown, soft puffy bread which is elastic and chewy (4). Sour curd is sometimes added to the dough in Bhatura cooked by the traditional method to reduce the fermentation time and consequently influence the microbial profile of the total fermentation process. Fried sourdough bread (bhatura) with an elevated amount of γ-aminobutyric acid (GABA) was produced using lactic acid bacteria (LAB) (1).

Fermentation can be broadly defined as the process in which carbohydrate or carbohydrate-like compounds are broken down under anaerobic or aerobic conditions (4). Each fermented food is associated with a unique group of microflorae, which increases the level of protein,

vitamins, essential amino acids, and fatty acids. The deficiency of protein and vitamins is a major worldwide problem. In this regard, the production of fermented food will become increasingly important to the world's diet (3). In the fermentation of food, a complex mixture of carbohydrates undergoes modifications simultaneously under the action of a variety of microorganisms and enzymes present. Thus, the carbohydrate and carbohydrate-like materials undergo "Fermentation". Fermentation typically refers to the conversion of sugar to alcohol using yeast under anaerobic conditions (5).

Lactic acid bacteria (LAB) are a large group of closely related bacteria that have similar properties such as lactic acid production, which is an end product of the fermentation. LAB include *Lactobacillus, Lactococus, Streptococcus,* and *Leuconostoc* species (6). Lactic fermentation of bread dough improves the keeping quality and flavour of the baked products. It also enhances the palatability of bread made from low-grade flour and under-utilized cereals (7). The name millet is applied to numerous small-seeded grasses which originated in Asia or Africa (8). Barnyard millet (*Echinochloa* species) has become one of the most important minor millet crops in Asia, showing a firm upsurge in world production. It has received appreciable attention for its susceptibility to biotic and abiotic stresses, multiple harvests in a year, and rich in micronutrients, fibers, and phytochemicals than other major cereals (9). The carbohydrate present in barnyard millet is low and slowly digestible, which makes barnyard millet a natural gift for mankind (10). They are rich in dietary fiber, glycaemic load and have micronutrients like vitamin such as magnesium, iron, and vitamin B (8).

It is believed that the consumption of barnyard millet can possess various health benefits against diabetes mellitus, cardiovascular diseases, obesity, skin problems, cancer, and celiac disease (8, 11). The flour of barnyard millet is gluten-free and can be incorporated into the diet of celiac and diabetic patients (8).

11.2 Objectives:

- To develop Barnyard millet-based fermented food product & evaluate its organoleptic properties.
- To study the effect of fermentation time on the product.
- For the development of low gluten and glycemic index product.

11.3 Materials and Methods:

11.3.1 Raw Materials:

The present study was carried out in the Department of Food Science and Nutrition, Yuvaraja's College (Autonomous), Mysuru.

The raw materials to prepare Barnyard millet Bhatura i.e., barnyard millet flour, maida flour, curd, semolina, salt, sugar, oil, baking soda, and baking powder, were procured from a local grocery shop in Mysuru.

11.3.2 Method of Preparation:

Bhatura was prepared by mixing the barnyard millet flour (BMF) & maida, semolina, sugar, salt, baking powder, baking soda, curd, oil, and water in a bowl making it a consistent dough and adding hot oil and mix again then tuck the dough and the smear oil over its surface and close the bowl with a muslin cloth and leave to rest for 2 hrs.

Make equal-sized balls out of dough and flatten them to obtain uniform thickness and deep-fry it till they become golden brown and crisp.

11.3.3 Sensory Analysis of Prepared Bhatura:

Sensory evaluation was carried out to determine the acceptability of various attributes such as appearance, taste, texture, colour, flavour, and overall acceptability.

The product was evaluated by taking the average score of the 30 semi-trained panellists by using a 9-point hedonic scale (12).

11.3.4 Standardization of Fermentation Time of the Dough:

The selected formulation of bhatura (B3) dough was fermented for different timings like 1, 2, 3, 4 h and was standardized for the fermentation time.

11.3.5 Nutritional Analysis of Prepared Bhatura:

Standard A.O.A.C. (1980) method was used to determine the Nutritional composition of selected variation (B3) of barnyard millet bhatura and control.

The moisture content was estimated by using a hot air oven at 98 to 100˚ C, Protein content was estimated by determining total nitrogen content using the standard Micro - Kjeldhal method, ash % was estimated by high-temperature incineration using a muffle furnace and fat content was estimated by the Soxhlet method.

The crude fiber content was estimated by a crude fiber analyser. The carbohydrate content was obtained by subtracting from 100 with the sum of values of moisture, protein, fat, and ash content per 100 g of the sample.

Minerals like Calcium, iron, and phosphorous were analysed using inductively coupled plasma mass spectrometry (ICPMS). These methods give good precision and accuracy [13].

11.3.6 Statistical Analysis:

Each sample was analysed in triplicates. The data obtained were analysed statistically using standard methods given by Snedecor and Cochran [14] and by Duncan's multiple range test with the $p \leq 0.05$ consider to be significant [15].

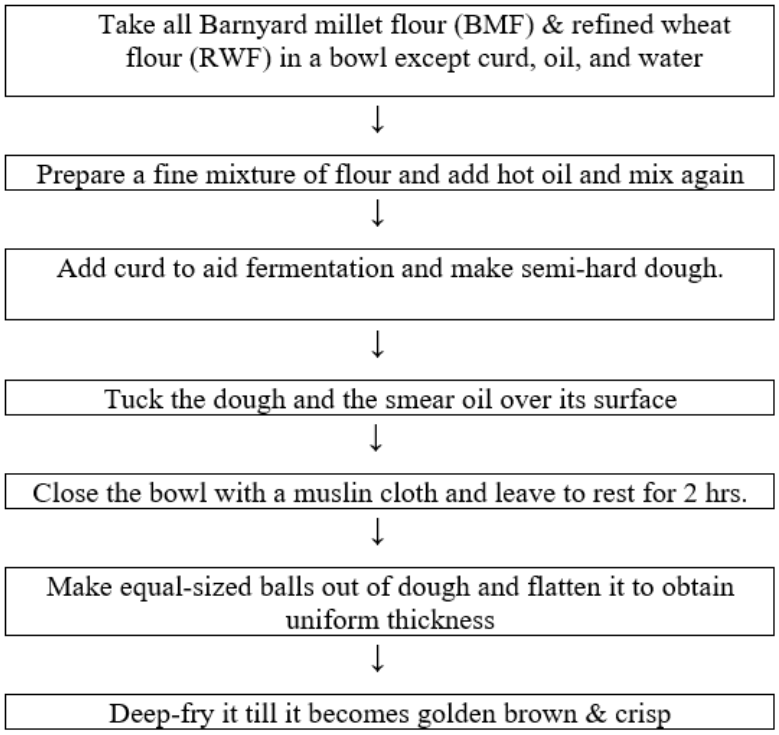
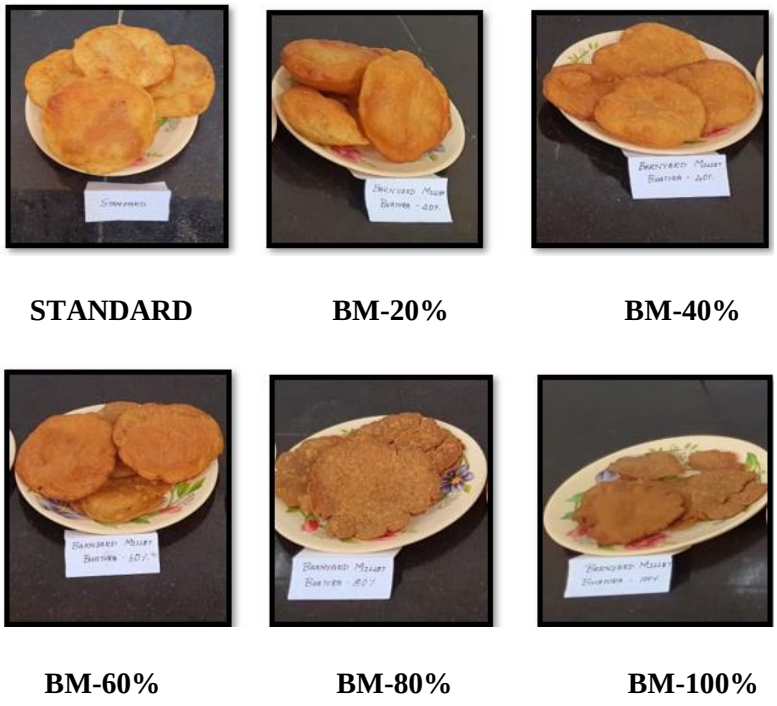


Figure 11.1: Flow Chart of preparation of Bhatura

11.4 Formulation of the Product:

Table 11.1: Standardization of Formula (ingredients g/100gm) for preparation of barnyard millet flour bhatura

Ingredients	B1 (Standard)	B2 (20%)	B3 (40%)	B4 (60%)	B5 (80%)	B6 (100%)
Barnyard millet flour (BMF)	100	80	60	40	20	0
Maida	0	20	40	60	80	100
Semolina	15	15	15	15	15	15
Sugar	2.5	2.5	2.5	2.5	2.5	2.5
Salt	2	2	2	2	2	2
Baking powder	1.25	1.25	1.25	1.25	1.25	1.25
Baking soda	1.25	1.25	1.25	1.25	1.25	1.25
Curd	30	30	30	30	30	30
Oil	3	3	3	3	3	3
Water	30	30	30	30	30	30



STANDARD **BM-20%** **BM-40%**

BM-60% **BM-80%** **BM-100%**

Figure 11.2: Different variations of bhatura developed from barnyard millet flour in comparison with maida flour bhatura.

Figure 11.3: Barnyard Millet Incorporated bhatura Prepared by Varying Fermentation Time

11.5 Results and Discussion:

11.5.1 Sensory Evaluation of Barnyard Millet Incorporated Bhatura:

The study was undertaken to prepare the barnyard bhatura by partially replacing maida with barnyard millet flour. The data pertaining to the effect of the incorporation of various levels of barnyard flour (20, 40, 60, 80, and 100%) on sensory attributes of bhatura and the results are shown in Table 11.2. The scores obtained for all sensory attributes for B2 and B3 had almost similar to the control, B4, B5 B6 showed decreased scores and were less acceptable compare to other variations. B3 had the highest sensory score compared with all the other variation.

Table 11.2: Sensory evaluation of different variations of bhatura developed from barnyard millet with partial replacement of maida flour. Values are mean ± SD, $p \leq 0.05$ (Holm Sidak method), n=30

Attributes	B1 (Standard)	B2 (20%)	B3 (40%)	B4 (60%)	B5 (80%)	B6 (100%)
Appearance	9 ± 0	8.6 ± 0.5	**8.8 ± 0.5**	7.6 ± 0.4	6 ± 0.5	5.6 ± 0.7*
Colour	9± 0	8.7 ± 0.4	**8.9 ± 0.4**	7.6 ± 0.5	6.3 ± 1.1	5.8 ± 0.8*
Texture	8.7 ± 0.5	8.6 ± 0.5	**8.7 ± 0.4**	7.6 ± 0.4	5.7 ± 0.8*	5.8 ± 0.7
Taste	8.6 ± 0.7	8.6 ± 0.6	**8.9 ± 0.5**	7.4 ± 0.5	5.6 ± 0.6*	5.8 ± 0.5
Flavour	8.9 ± 0.6	8.6 ± 0.6	**8.8 ± 0.4**	7.4 ± 0.5	5.8 ± 0.6	5.8 ± 0.5*
Overall acceptability	8.9 ± 0.4	8.6 ± 0.6	**8.8 ± 0.4**	7.4 ± 0.5	5.8 ± 0.5*	6.2 ± 0.5

11.5.2 Standardization of Fermentation Time:

The most acceptable variation 40 % (B3) was further prepared by varying their fermentation time. The data pertaining to the effect of different fermentation times on barnyard millet bhatura and the results are shown in Table 11.3.

The result showed that the optimum fermentation time for barnyard millet incorporated bhatura was found to be 3h and acceptable up to 40 %. The proximate composition of accepted barnyard millet bhatura B3 (40 %) (3 h fermentation time) and that of control were analysed and the results of the same are shown in Table 11.3.

Table 11.3: Sensory scores of bhatura prepared with BM incorporated for different fermentation times. Values are mean ±, n=15 *$p < 0.05$ (Holm sidak method), n=30.

Attributes	1 hr	2 hr	3 hr	4 hr
Appearance	7.2 ± 0.6*	7.3 ± 0.4	**7.9 ± 0.7**	7.3 ± 0.4
Colour	7.1 ± 0.3*	7.6 ± 0.6	**7.9 ± 0.7**	7.2 ± 0.4
Texture	6.6 ± 0.5*	7.5 ± 0.8	**7.8 ± 0.8**	7.4 ± 0.6
Taste	7 ± 0.7*	7.6 ± 0.6	**7.8 ± 0.6**	7.2 ± 0.4
Flavour	7.6 ± 0.7	7.5 ± 0.6	**7.9 ± 0.5**	7.2 ± 0.4*
Overall acceptability	7.5 ± 0.6	7.5 ± 0.6	**7.7 ± 1.0**	6.9 ± 0.5*

11.5.3 pH Value:

The pH of the BM Bhatura dough was measured using the pH paper. pH value of Bhatura dough at different fermentation times varied from 6.0 to 7.0 (1, 2, 3, and 4 hrs) and the selected variation of the fermentation time was 3 hrs. The initial pH of the dough was 7.0, as fermentation occurred the pH was reduced to 6.0 even at different fermentation times.

Acidic pH in Bhatura is primarily due to the fermentation process which is from the lactic acid bacteria. Lactic acid fermentation is mainly responsible for the taste, texture, and acidic pH and also it helps in the leavening of the dough of the Bhatura.

Table 11.4: pH Value

Variations	Initial pH	pH at 1h	pH at 2h	pH at 3h	pH at 4h
BM1 (Control)	7	6	6	6	6
BM2 (20%)	7	6	6	6	6
BM3 (40%)	**7**	**6**	**6**	**6**	**6**
BM4 (60%)	7	6	6	6	6
BM5 (80%)	7	6	6	6	6
BM6 (100%)	7	6	6	6	6

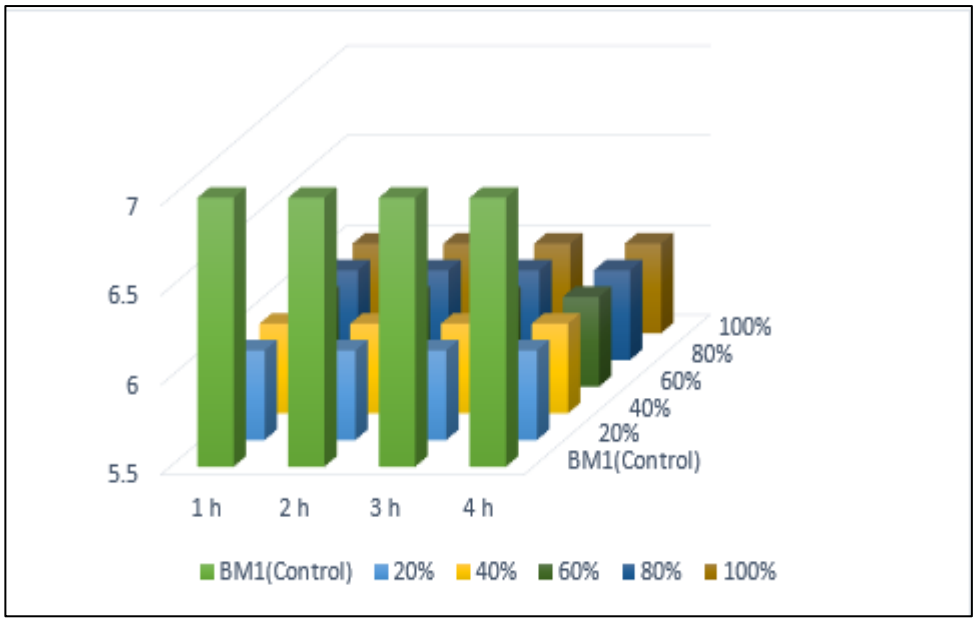


Figure 11.4: Determination of pH of BM Incorporated Bhatura Dough

11.5.4 Proximate Composition of prepared bhatura

The proximate composition of accepted barnyard bhatura (B3) and that of control were analysed and the results of the same are shown in Table 11.5.

The moisture content of all the variations of bhatura was similar.

The values of protein and fat content were higher in B3 than that of control, whereas carbohydrate was less. However, protein, fiber, ash, iron, calcium, and phosphorus content were increased in barnyard flour bhatura.

Table 11.5: Proximate Composition of selected variation (B3) of bhatura developed from Barnyard millet flour with partial replacement of maida flour. Values are mean ± SD, p ≤0.05 (Holm sidak), n=30

Nutrients	Standard Bhatura	B3(40%)
Energy (Kcal)	434.06±0.12	428.42±0.10
Carbohydrate (g)	64.04±0.34	60.39±0.22
Protein (g)	8.27±0.37	10.39±0.29
Fat (g)	15.9±0.96	16.3±0.33
Crude Fiber (g)	0.11±0.1	0.31±0.18
Ash (g)	1.2±0.16	1.6±0.34
Moisture (%)	10.48±0.12	11.01±0.32
Iron (mg)	5.5±0.50	13.22±0.3*
Phosphorus(mg)	176.8±0.18	190.8±0.11*

11.6 Conclusion:

Bhatura is a popular traditional sourdough bread commonly served as a midday meal or as breakfast in northern and eastern parts of the Indian subcontinent. Here an attempt was made to develop bhatura using barnyard millet flour. Maida is a high glycaemic food where the barnyard millet is high in protein, fiber, and micronutrients and low in carbohydrate content which makes it low glycaemic food. Barnyard millet is gluten-free and rich in iron, calcium, minerals, and vitamin B complex. In our study partial replacement of maida flour with 40% of barnyard flour and which was fermented for 3 h had the highest acceptability in the sensory scores. When compared to the control, the selected barnyard millet had significantly increased levels of the proximate nutrient content of protein, Dietary fiber, Iron & Phosphorus. Moreover, it reduced the carbohydrate content of Bhatura making it low in glycemic index. Therefore, barnyard bhatura is a better choice for celiac disease and it also has a low glycemic index compared to standard bhatura. As this bhatura contains curd which has probiotics or live bacteria like *Lactobacillus* and *Bifidobacterium.* These bacteria strengthen the digestive system and reduce conditions like bloating, irritable bowel syndrome, dysentery, constipation, gastrointestinal disorders, and diarrhea.

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12. Effect of Fermentation Time on Sensory Attributes of Sorghum (*Sorghum bicolor*) Selroti

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Abstract:

Fermentation is one of the oldest biotechnological processes that preserves food, improves nutritional value and enhances sensory attributes. Selroti is a spongy, ring-shaped, rice-based, fermented dish that is indigenous to Nepal. It has a good number of digestible proteins. Lactobacilli, Pediococcus, Enterococci and Leuconostoc are the principal microorganisms present in selroti. Sorghum (Sorghum bicolor) popularly called jowar, is the "king of millets". Sorghum is a member of grass family (Poaceae). It is a gluten-free-Nutri-cereal and contains significant levels of iron, starch, protein, antioxidant and phenolic compound. It is the ideal food for diabetics because starches and sugars in sorghum are released more slowly than in other millet. In this study, Selroti S1, S2, S3, S4,

S5 and S6 containing jaggery, refined wheat flour, spices with different compositions of sorghum and rice flour (0%, 20%, 40%, 60%, 80%, and 100%) were formulated. Selroti prepared with 60% sorghum and fermented for 6 h had the highest scores in terms of sensory scores. Moreover, Selroti prepared from sorghum had more protein, fiber, and phosphorus.

Keywords:

Pediococcus, Leuconostoc, antioxidant, gluten-free, jowar.

12.1 Introduction:

Sel-roti is a delicious energy-dense food with an intermediate shelf-life. It is a well-liked rice-based fermented, deep-fried food item that is fashioned like a ring and eaten in Sikkim and the Darjeeling hills in India, Nepal, and Bhutan (1). Sel-roti is a Nepali word for ring-shaped rice-based bread that is traditionally prepared for religious festivals and special occasions. *Lactobacilli*, *Pediococcus*, *Enterococci* and *Leuconostoc* are the principal microorganisms (2). Millets, commonly referred to as ancient grains, are a class of extremely healthy and adaptable grains that have been consumed for many centuries (3).

They are commonly referred to as "Nutri-cereals" since they contain the majority of the nutrients needed for the human body to function normally. Sorghum (*Sorghum bicolor*), also known as jowar or, is known as the "king of millets" (4).

It is renowned for its exceptional agronomic performance, or its capacity to adapt to a range of settings and grow. Sorghum has a variety of health advantages due to its distinctive phenolic profile, including a reduction in oxidative stress and cancer prevention (5).

12.2 Objective:

- To develop Sorghum-based Selroti by partially replacing rice with sorghum
- To evaluate the organoleptic acceptability of the developed product
- To determine the optimum fermentation time on the developed product

12.3 Materials and Methods:

12.3.1 Raw Materials:

Raw materials such as sorghum, rice, jaggery, refined wheat flour, cardamom, cinnamon, clove, coconut powder, and milk were procured from the local market of Mysuru.

12.3.2 Methods of Preparation:

The rice and sorghum were sorted, washed, and soaked in coldwater for overnight at ambient temperature. Water is then decanted from the rice, spreadover a cotton cloth and dry it. Rice that has been soaked is ground into a coarse powder and sieved.

The rice flour is then well combined with 25 g of refined wheat flour, 25 g of jaggery, and 2.5 g of spices including cardamom, cloves, coconut powder, and cinnamon. Boiled milk is added, kneaded into a soft dough, and then easily poured into batter. The batter is allowed to organically ferment for 3 hours at room temperature (20 28°C). In a cast iron frying pan, the oil is heated. A continuous ring of the fermented batter is squeezed out by hand, dropped into hot edible oil, and fried till golden brown before being drained from the hot oil. Selroti is offered as candy and is deep-fried (2).

12.3.3 Optimization of Batter Fermentation:

The batter was allowed to ferment for different periods (3, 6, 9, 12 h) in a stainless-steel vessel. No effort was made to control the temperature during the process of fermentation.

12.3.4 pH and Volume:

For the different fermentation times and blend ratios of the Selroti batter properties viz. volume raised, pH was studied. The volume was recorded at 3, 6, 9 and 12 h with the help of measuring cylinder. The pH of the batter at different fermentation time was recorded initially and at the end of fermentation using pH paper.

12.3.5 Sensory Analysis of Prepared Selroti:

Sensory evaluation was carried out to determine the acceptability of various attributes such as appearance, taste, texture, color, flavor and overall acceptability.

The product was evaluated by taking the average score of semi- trained panelists by using a 9-point hedonic scale.

12.3.6 Nutritional Analysis of Prepared Selroti:

Standard A.O.A.C (1980) methods were used to determine the nutritional composition of selected variation S4 (6 h) of selroti and control.The moisture content was estimated by using a hot air oven at 98 to 100˚ C, and protein content was estimated by determining total nitrogen content using the standard Micro- kjeldhal method, ash % was estimated by high temperature incineration using muffle furnace and fat content was estimated by the Soxhlet method. The crude fiber content wasestimated by crude fiber analyzer. The carbohydrate content was obtained by subtracting from 100 with the sum of values of moisture, protein, ash and fat content per 100 g of the sample. Minerals like calcium, phosphorous and iron were analyzed using inductively coupled plasma mass spectrometry (ICPMS). These methods gave a good precision and accuracy (6).

12.3.7 Statistical Analysis:

Each sample was analyzed in triplicates. The data obtained was analyzed statistically using standard methods given by Snedecor and Cochran and by Duncan's multiple range test with the probability $p \leq 0.05$ considered to be significant (7).

12.4 Formulation of the Product:

Table 12.1: Formulation of the products (ingredients g/100 g) for preparation of sorghum selroti

Ingredients	S1 (Standard)	S2 (20%)	S3 (40%)	S4 (60%)	S5 (80%)	S6 (100%)
Rice (g)	100	80	60	40	20	-
Sorghum (g)	-	20	40	60	80	100
Refined wheat flour (g)	25	25	25	25	25	25
Jaggery (g)	25	25	25	25	25	25
Coconut powder (g)	1.25	1.25	1.25	1.25	1.25	1.25
Cardamom (g)	0.5	0.5	0.5	0.5	0.5	0.5
Cinnamon (g)	0.5	0.5	0.5	0.5	0.5	0.5
Clove (g)	0.25	0.25	0.25	0.25	0.25	0.25
Milk (ml)	50	50	50	50	50	50

Rice + Sorghum
↓
Wash in cold water and soak for 6 hours
↓
Decant the water, pound and sieve to get flour
↓
Add refined wheat flour, jaggery and spices (cardamom, cinnamon, clove, coconut powder)
↓
Add the boiled milk to the mixture
↓
Mix thoroughly to make the batter
↓
Ferment at 28 °C for 3 hours
↓
Squeeze the batter in to ring shape
↓
Deep fry it until it becomes golden brown

Figure 12.1: Flow chart for preparation of Sorghum Selroti

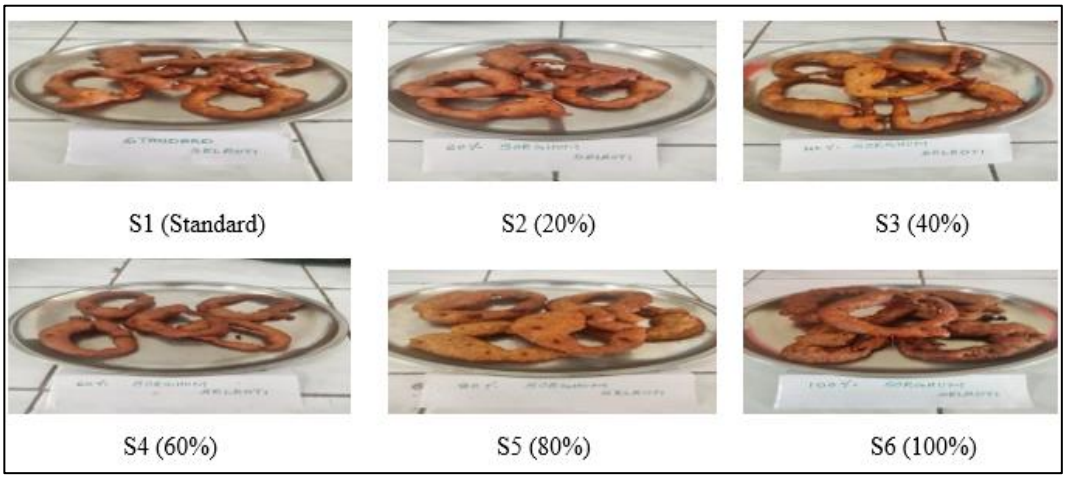

Figure 12.2: Different variations of Selroti developed from Sorghum in comparison with rice Selroti

12.5 Result and Discussion:

12.5.1 Sensory evaluation of Sorghum Selroti:

The study was undertaken to prepare Sorghum-based Selroti by partially replacing rice with Sorghum. The data pertaining to the effect of incorporation of various levels of Sorghum (20, 40, 60, 80 and 100%) on sensory attributes of Selroti and the results are shown in Table 12.2. The scores obtained for S2, S3 and S4 were almost similar on par with the control. S5 and S6 showed decreased scores and were less acceptable compared to other variations.

The acceptable S4 variation was incubated for a different fermentation time to study the optimum fermentation time of sorghum incorporated selroti.

Table 12.2: Sensory evaluation of different variation of Selroti developed from Sorghum with partialreplacement of rice.

Attributes	S1 (Standard)	S2 (20%)	S3 (40%)	S4 (60%)	S5 (80%)	S6 (100%)
Appearance	8.52±0.53	8.26±0.48	8.35±0.51	8.46±0.69	7.9±0.41*	7.8±0.24*
Color	8.49±0.14	8.34±0.18	8.21±0.58*	8.44±0.11	8.013±0.80	7.91±0.58*
Texture	8.31±0.86	8.22±0.42	8.11±0.28	8.28±0.77	8.1±0.14	8.24±0.05
Taste	8.55±0.23	8.38±0.37	8.40±0.15	8.53±0.59	7.93±0.52*	7.85±0.63*
Flavor	8.40±0.61	8.33±0.67	8.36±0.08	8.38±0.21	7.83±0.16	7.72±0.72*
Overall acceptability	8.51±0.56	8.28±0.21	8.29±0.23	8.48±0.33	7.86±0.28	7.67±0.48*

Values are mean ± SD (n=30), *p value ≤0.05 (Holm Sidak method)

12.5.2 pH:

pH value of Selroti batter at different fermentation period ranged from 5.0 to 6.0 (3, 6, 9,12 hours). Initial pH was 6.0, as fermentation occurred the pH was 5.0 throughout process even after varying the fermentation time.

There is an increase in acidity level, i.e., decrease in the pH value. This is mainly due the development of Lactic acid bacteria producing lactic acid which lowers the pH, and the production of carbon dioxide, which leavens the batter.

Table 12.3: pH of Selroti batter

Variations	Initial pH	pH after 3 h	pH after 6 h	pH after 9 h	pH after 12 h
S1 (Control)	6	5	5	5	5
S2 (20%)	6	5	5	5	5
S3 (40%)	6	5	5	5	5
S4 (60%)	6	5	5	5	5
S5 (80%)	6	5	5	5	5
S6 (100%)	6	5	5	5	5

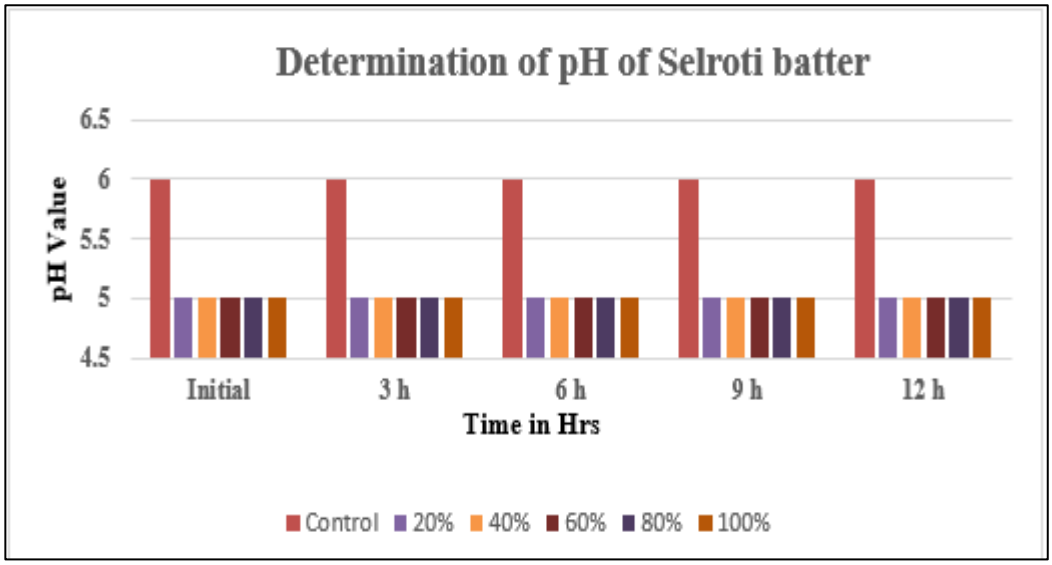

Figure 12.3: pH of Selroti Batter

12.5.3 Volume:

Initial volume of the batter was 80 ml and an increase in 10 ml of volume occurred for all the variations. This increased volume of selroti batter is due to increase incorporation of the lactic acid bacteria into the batter during fermentation and entrapment of air.

Table 12.4: Determination of volume of Selroti batter

Variations	Initial volume (ml)	Volume after 3 h (ml)	Volume after 6 h (ml)	Volume after 9 h (ml)	Volume after 12 h (ml)
S1 (Control)	80	90	90	90	90
S2	80	90	90	90	90
S3	80	90	90	90	90
S4	80	90	90	90	90
S5	80	90	90	90	90
S6	80	90	90	90	90

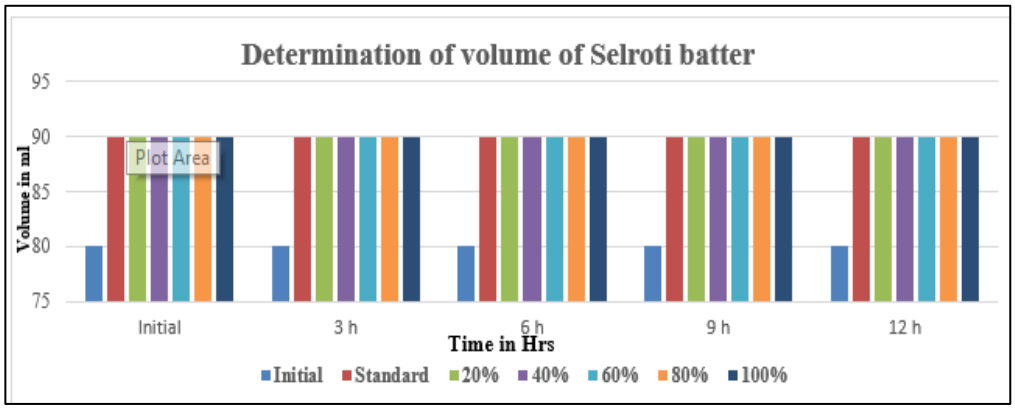

Figure 12.4: Volume of Selroti Batter

12.5.4 Optimization of Batter Fermentation:

The data pertaining to the effect of different fermentation times of Sorghum (3 h, 6 h, 9 h, 12 h) on sensory attributes of Selroti and the resultsare shown in Table 12.5. Variation S4 (6 h) was more acceptable in terms of sensory attributes.

Table 12.5: Sensory evaluation of different variation of Selroti developed from Sorghum with partial replacement of rice by varying fermentation time.

Attributes	3 h (Control)	S4 (6 h)	S4 (9 h)	S4 (12 h)
Appearance	8.46±0.69	9±0	7.86±0.07	7.26±0.45
Color	8.44±0.11	8.86±0.35	7.6±0.63*	7.33±0.48*
Texture	8.28±0.77	8.66±0.48	7.82±0.56	7.4±0.05*
Taste	8.53±0.5	8.73±0.45	7.8±0.86	7.53±0.5*
Flavor	8.38±0.21	8.86±0.35	7.93±0.45	7.4±0.03*
Overall acceptability	8.48±0.33	8.91±0.25	7.73±0.59	7.5±0.1*

S4 (3 h) S4 (6 h) S4 (9 h) S4 (12 h)

Figure 12. 5: Sorghum incorporated Selroti developed by varying Fermentation time

12.5.5 Nutritional Analysis:

The proximate composition of accepted Sorghum selroti S4 (6 h) and that of the control were analyzed and the results of the same are shown in Table 12.4. The moisture content of all variations of Selroti was similar. The values of protein were higher in S4 (6 h) thanthat of control, whereas carbohydrate was less. However, fiber, ash, iron, calcium and phosphoruscontent were increased in Sorghum selroti.

Table 12.4: Proximate Composition of selected variation S4 (6h) of Selroti developed fromSorghum with partial replacement of rice.

Nutrients/100 g	Control Selroti (S1)	S4 (6 h)
Energy (Kcal)	429.71±0.17	439.64±0.21*
Carbohydrate (g)	59.47±0.14	57.85±0.08
Protein (g)	7.51±0.08	7.56±0.12
Fat (g)	18.31±0.11	17±0.04
Fiber (g)	2.90±0.01	3.49±0.16*
Ash (g)	0.42±0.03	0.5±0.17
Moisture (%)	11.39±0.25	13.6±0.01*
Phosphorous (mg)	92±0.03	133.2±0.18*
Iron (mg)	0.28±0.02	5±0.02*
Calcium (mg)	6.44±0.12	18±0.2*

Values are mean SD, p ≤0.05 (Holm sidak), n=3

12.6 Conclusion:

Selroti is a rice-based fermented food that gets its sponginess via fermentation, which also rises batter volume and folic acid volume. Here we attempted to develop selroti using sorghum Minerals, fiber and antioxidant are abundant in sorghum. In our study partial replacement of rice with 60% of sorghum was acceptable. With maximum scores for S4 (6h),

standardizing the fermentation time for a selected variation led to greater acceptance in terms of sensory qualities. As a result of poor fermentation, S4 (9 h) had lowest sensory scores and the last variation was also not good due to hyper-fermentation. Sorghum Selroti had increased level of Protein, Dietary fiber, Iron, Calcium and Phosphorous. Moreover, it reduced the carbohydrate content. The optimumfermentation time for Sorghum incorporated Selroti was found to be 6 h and acceptable up to 60%.

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13. Effect of Fermentation on Sensory Properties of Idli Prepared by Incorporation of Proso Millet (*Panicum Miliaceum*)

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Abstract:

Idli is a traditional breakfast food of India especially popular in South India. It is a fermented food product, which is prepared by steaming fermented parboiled rice and black gram dhal batter. Black gram dhal is the major component in the fermentation of batter. The lactic acid bacteria are responsible for the fermentation of batter. This process increases the nutritive value of idlis, gives a soft and spongy texture, and also improves digestibility. Proso millet is rich in proteins, dietary fiber, polyphenols, vitamins, and minerals such as iron, zinc, copper, and manganese.

In this study, different varieties of idlis were prepared by incorporating different proportions of proso millet with parboiled rice. Six formulations (P1, P2, P3, P4, P5, P6) containing different compositions of proso millet (0%, 20%, 40%, 60%, 80%, and 100%) along with rice and black gram dhal were used to prepare Idli. These formulations were analyzed for their sensory attributes and sensory scores were highest for P4 idli. The fermentation time of proso millet incorporated idli P4 (60%) was optimized by varying fermentation time (6 – 24 h). The sensory scores were highest for the product which was fermented for 6 h. The proximate nutritional composition of the best-accepted variation was analyzed by the standard AOAC method. The protein, phosphorous, iron, and fiber contents were increased in millet-incorporated idlis. This study documents the role of fermentation in batter characteristics and quality of idli in terms of nutrition and sensory attributes.

Keywords:

Fermentation time, lactic acid bacteria, millet incorporation, dietary fiber.

13.1 Introduction:

Cereal/legume-based foods are the most commonly consumed foods worldwide. Among the cereal/legume-based fermented foods, Idli is one of the most widely consumed and highly popular traditional food of India, also known as 'Rice cake'. Idli is a naturally fermented and steamed food product that is made from batter prepared from rice and black gram dhal. The specialty of black gram in idli preparation is owing to the mucilaginous material present in it. This mucilaginous principle helps in the retention of carbon dioxide evolved during fermentation (1, 2, 3).

Two significant changes occurring in idli batter fermentation are leavening and acidification. Fermentation improves texture, taste, flavor, digestibility, and nutritive value. Its digestibility improves as the complex sugars are converted into simple sugars during fermentation. It also encourages a healthy gut microflora (4). Idli has good nutritive value and makes an important contribution to the diet as a source of protein, calories, and vitamins especially B-complex vitamins compared to raw unfermented ingredients (5, 6).

Millets are small-seeded cereal grains commonly grown in Asia, African countries, and parts of Europe and consumed as a staple food among the majority of people in the arid and semiarid tropics of the world (7). Proso millet *(Panicum miliaceum)* is the oldest cultivated millet crop and is often cultivated in hot and dry conditions. It is superior to rice and wheat because it provides protein, minerals, and vitamins to the poor where the need for such nutrients is in high demand (8).

Proso millet is rich in nutraceuticals like dietary fiber, omega-3 fatty acids, phenolics, and flavonoids which play a potential role in the treatment of degenerative diseases and metabolic disorders. Proteins from proso millets also improve cholesterol metabolism (9).

The food industries in Europe and North America are interested in proso millet for its potential health benefits for humans and also for its mild flavor, light color, and gluten-free characteristics (10).

13.2 Objectives:

- To develop Idli from proso millet
- To evaluate the organoleptic acceptability of the developed product
- To study the effect of fermentation time on product

13.3 Materials and Methods:

13.3.1 Raw Materials:

The present study was carried out in the Department of Food Science and Nutrition, Yuvaraja's College, University of Mysore, Mysuru.

The raw materials such as Parboiled rice, Proso millet, Black gram dhal, and salt were procured from a local grocery shop in Mysuru.

13.3.2 Methods:

A. Preparation Of Proso Millet (PM) Idli: Idli was prepared by replacing parboiled rice with PM in different proportions. Parboiled rice, PM, and black gram dhal were soaked for 4 hours. The mixture of parboiled rice and PM was grounded into a coarse slurry and black gram dhal was into a fine paste. After that slurry and paste were combined into a thick batter and mixed well by adding salt. The batter was allowed to ferment for 12 hours in a warm place (34-37 °C). Then the fermented batter was poured into small cups of idli cooker and steamed for 20 minutes (11).

B. Sensory Analysis of Idli: Sensory evaluation of idli was done for the sensory attributes such as appearance, color, texture, taste, flavor, and overall acceptability to determine the acceptability of idlis. The product was evaluated by 20 semi-trained panelists using a 9-point hedonic scale method.

C. Optimization of Batter Fermentation: The batter of selected variation (P4) was allowed to ferment for different periods (6 h, 12 h, 18 h, and 24 h).

D. Ph and Volume: For the different fermentation times of selected variation (P4) batter, the batter properties viz., pH and volume were studied. pH was recorded initially and at the end of fermentation using pH paper. Initial batter volume and volume raised after fermentation was measured using a measuring cylinder.

E. Nutritional Analysis of Idli: Standard AOAC (1980) method was used to determine the nutritional composition of selected variation (P4) of PM and control. The moisture content was estimated by using the hot air oven method (98 to 100 ˚C), Protein content was estimated by determining total nitrogen content using the standard Micro-Kjeldahl method, ash % was estimated by high-temperature incineration using a muffle furnace and fat content was estimated by Soxhlet method. The crude fiber content was estimated by using a crude fiber analyzer. The carbohydrate content was obtained by subtracting the sum of values of moisture, protein, fat, and ash content (per 100 g of the sample) with 100. Minerals like calcium, iron, and phosphorous were analyzed using inductively coupled plasma mass spectrometry (ICPMS). These methods give good precision and accuracy (12, 13).

F. Statistical Analysis: Each sample was analyzed in triplicates. The data obtained were analyzed statistically using standard methods given by Snedecor and Cochran (14) and by Duncan's multiple range test with the $p \leq 0.05$ consider to be significant (15).

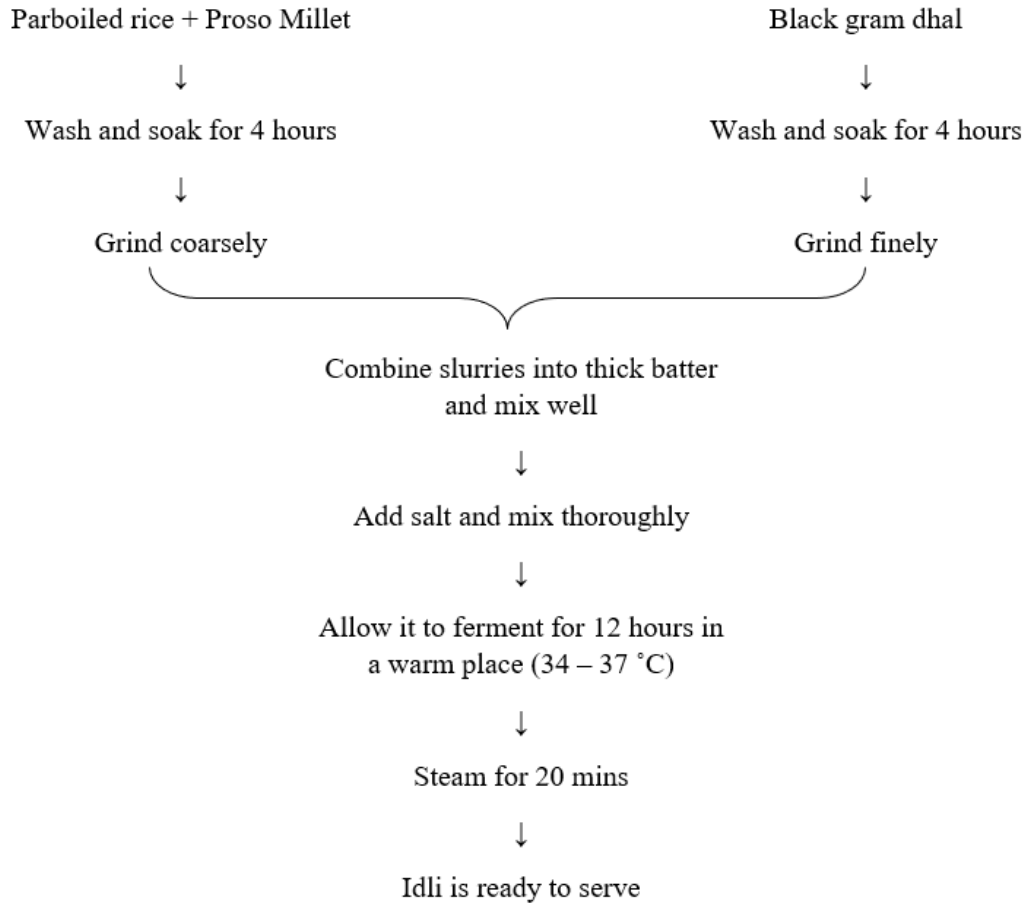

Figure 13.1: Flow Chart for Preparation of PM Idli

13.4 Formulation of the Product:

Table 13.1: Formulation of ingredients (g/100 g) for preparation of PM idli

Ingredients	P1 (Standard)	P2 (20%)	P3 (40%)	P4 (60%)	P5 (80%)	P6 (100%)
Proso millet (g)	-	20	40	60	80	100
Parboiled rice (g)	100	80	60	40	20	-
Black gram dhal (g)	50	50	50	50	50	50
Salt (g)	2	2	2	2	2	2

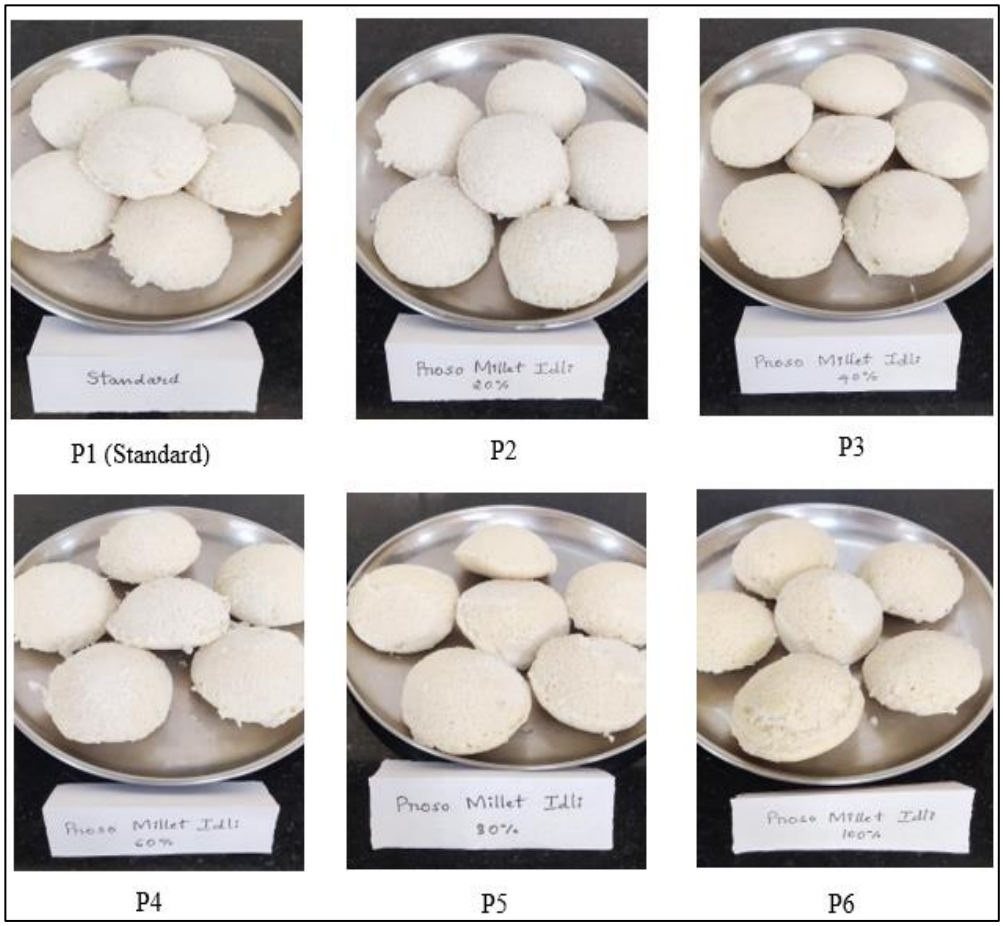

Figure 13.2: Different variations of Idlis developed from PM in comparison to Parboiled rice idli

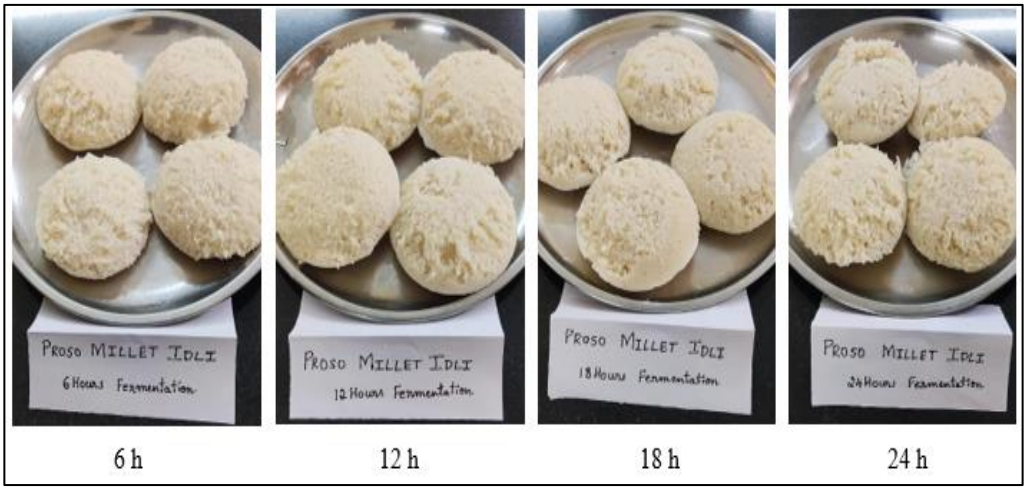

Figure 13.3: PM incorporated idli prepared by varying fermentation time

13.5 Results and Discussion:

13.5.1 Sensory Evaluation of PM Incorporated Idli:

This study was undertaken to prepare Idli by partially replacing rice with proso millet. The data about the effect of the incorporation of various levels of proso millet (20 %, 40 %, 60 %, 80 %, and 100 %) on the sensory attributes of Idli and the results are shown in Table No. 13.2. The results showed that P4 (60 %) variation had more acceptability. The scores obtained for sensory attributes for P2 were almost similar on par with the control. P5 obtained least scores and had less acceptability compared to all other variations.

Table 13.2: Sensory evaluation of different variations of Idli developed from the incorporation of PM with Parboiled rice, Values are mean ± SD, $p \leq 0.05$ (Holm Sidak method), n=20

Attributes	P1 (Standard)	P2 (20%)	P3 (40%)	P4 (60%)	P5 (80%)	P6 (100%)
Appearance	8.60±0.50	7.80±0.56	7.33±0.48	8.06±0.25	7.06±0.25*	6.93±0.45*
Color	8.66±0.48	7.73±0.59	7.06±0.45*	7.93±0.25	6.33±0.48*	6.73±0.70*
Texture	8.06±0.45	7.33±0.89	6.73±0.45*	7.73±0.45	6.20±0.41*	6.20±0.41*
Taste	8.26±0.70	7.33±0.72	6.73±0.45*	7.66±0.48	6.26±0.45*	6.26±0.45*
Flavor	8.20±0.67	7.33±0.61	6.93±0.25*	8.06±0.25	6.33±0.48*	6.33±0.48*
Overall acceptability	8.26±0.59	7.4±0.63	6.80±0.41*	7.93±0.45	6.33±0.48*	6.26±0.45*

13.5.2 Optimization of Fermentation Time:

The most accepted variation P4 (60 %) was optimized for the fermentation time by varying the fermentation time (6 to 24 hours). The data about the effect of different fermentation times on proso millet idli and the results are shown in Table No. 13.3. The result showed that the optimum fermentation time for proso millet incorporated idli was found to be 6 hours. Fermentation time was decreased from 12 hours to 6 hours because of the incorporation of proso millet.

Table 13.3: Sensory scores of Idli prepared with PM and parboiled rice incubated for different fermentation times, Values are mean ± SD, $p \leq 0.05$ (Holm Sidak method), n=20

Attributes	Standard	6 h	12 h	18 h	24 h
Appearance	8.60±0.50	8.26±0.5	7.80±0.4	7.53±0.6	7.20±0.5*
Color	8.66±0.48	8.06±0.7	7.46±0.6	7.46±0.6	7.20±0.5*
Texture	8.06±0.45	8.06±0.7	7.86±0.7	7.53±0.9	7.33±0.6
Taste	8.26±0.70	8.0±0.5	7.93±0.7	7.13±0.9	7.13±0.5
Flavor	8.20±0.67	7.93±0.5	7.86±0.5	7.06±0.8*	7.13±0.5*
Overall acceptability	8.26±0.59	7.93±0.4	7.86±0.6	7.13±0.9*	7.13±0.5*

13.5.3 pH Value:

The pH of the selected variant of PM idli (P4) batter was measured using pH paper for different fermentation times (6 to 24 hours). The initial pH of the batter was 6 and it was the same for all the variations.

pH was the same i.e., 6 in 6 hours of fermentation, and in 12 hours of fermentation, pH was decreased from 6 to 5. In 18- and 24-hour fermentation pH was decreased from 6 to 4, which shows the acidic nature this may be due to the increased activity of the lactic acid bacteria in a nutrient medium resulting in increased level of lactic acid.

Sugars are converted into lactic acid by the lactic acid bacteria present in the batter. Lactic acid fermentation is responsible for the sour taste and acidic pH of idli batter.

The acidic environment which is created by organic acids inhibits the growth of harmful bacteria and promotes the growth of beneficial bacteria. It also contributes to the characteristic texture and taste of idli.

Table 13.4: pH of PM incorporated (P4) idli batter fermented for different time periods

Variations	Initial pH	pH after fermentation
6 hours	6	6
12 hours	6	5
18 hours	6	4
24 hours	6	4

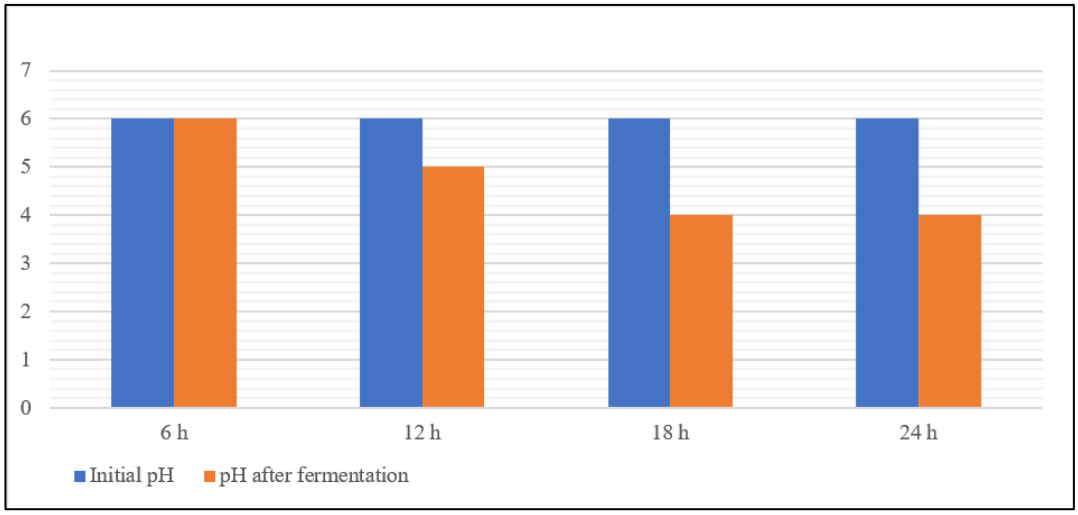

Figure 13.4: Determination of pH of PM Incorporated Idli batter

13.5.4 Volume:

The difference in volume was measured for the PM idli batter before and after fermentation. Batter was fermented for different time periods in a warm place (34 to 37 °C) (6 to 24 hours). The volume of the batter before fermentation was 60 ml and the volume after fermentation was 70 ml for 6 hours, 76 for 12 hours, 84 for 18 hours, and 92 for 24 hours of fermentation. As the fermentation time increases the volume of the batter increases. The volume raised because of the production of carbon dioxide gas by the microorganisms present in the batter. Gas gets trapped within the batter, forming air cells, and causes the batter to rise, resulting in the fluffy and soft texture of idli.

Table 13. 5: Volume of PM incorporated (P4) idli batter before and after Fermentation

Variations	Initial volume	Volume after fermentation
6 h	60	70
12 h	60	76
18 h	60	84
24 h	60	92

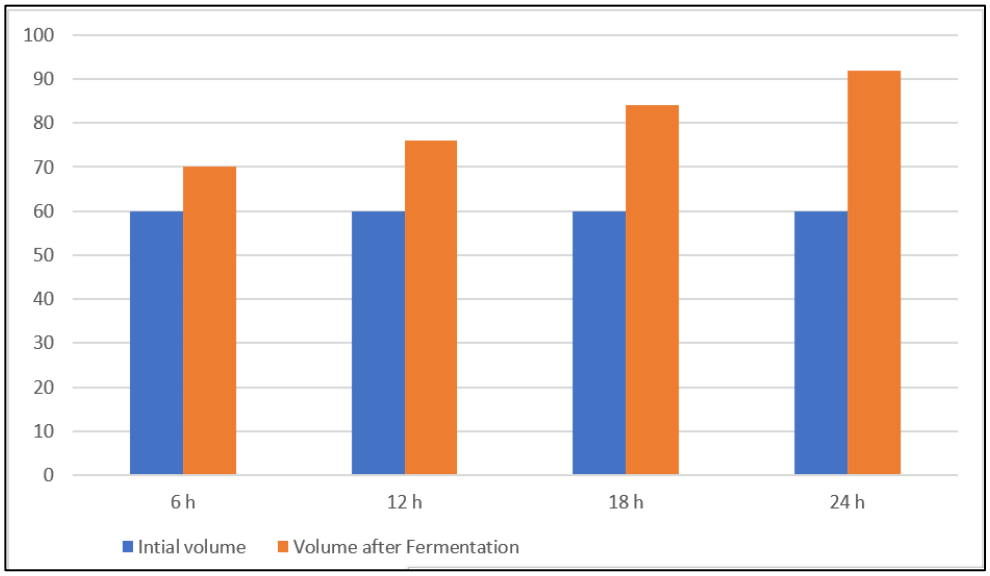

Figure 13. 5: Determination of Volume of PM incorporated idli batter (P4)

13.5.5 Nutrient Analysis:

The proximate composition of accepted PM idli P4 (60 %, 6 h fermentation time) and that of control were analyzed. The results are shown in Table No. 13.6. The values of protein, fat, and ash content were higher in P4 than that of the control, whereas carbohydrates and moisture were decreased. However, iron and phosphorus content were rich in proso millet idli.

Table 13.6: Proximate analysis of selected variation (P4) of PM incorporated idli,

Nutrients/100 g	Standard	PMI
Moisture (%)	24.03±0.15	20.36±1.58*
Carbohydrate (g)	60.90±0.95	56.52±0.55*
Protein (g)	11.81±1.10	18.64±0.49*
Fat (g)	0.84±0.35	1.97±0.28*
Crude fiber (g)	0.83±0.03	0.86±0.05
Ash (g)	1.59±0.02	1.65±0.06*
Energy (kcal)	298.4±0.19	318.37±0.18*
Iron (mg)	1.96±0.12	5.0±0.06*
Phosphorous (mg)	95.37±0.12	123.0±0.66*

Values are mean ± SD, $p \leq 0.05$ (Holm Sidak method), n=20

13.6 Conclusion:

Idli is an Indian traditional cereal/legume-based fermented product. In this study, the new product developed was Proso millet idli. Idli was chosen for this product development because of the wide acceptance of idli among consumers. Organoleptic evaluation of idli revealed that PM (60 %) with 6h fermentation time had high acceptance for its appearance color, texture, flavor, and taste among all variations. Fermentation helps in increased digestibility and nutritional value. Lactic acid bacteria are mainly responsible for fermentation which improves the characteristic texture, aroma, and taste of the idli. Millets are nutritionally superior crops. They are rich in minerals, vitamins, and proteins. It is gluten-free so ideal for gluten intolerant people. So, new value-added products can be developed from millets. The optimum fermentation time for Proso millet incorporated idli was found to be 6 h and acceptable up to 60 %. Sensory evaluation of Proso millet idli showed that it is highly acceptable to develop because of its improved sensorial characteristics, and nutritional and health benefits.

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14. Anti-Microbial Activity of *Rauwolfia Serptina* (*Sarpagandha*)

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Abstract:

Rauwolfia serpentina (RS) is a significant medicinal plant, also known as Sarpagandha. The Sarpagandha is a species of flowering plant in the family Apocynaceae which has been traditionally used in Ayurveda for many years to treat the variety of diseases. The drug Sarpagandha is cardio depressant, hypnotic and sedative. It is primarily used in preventing the various diseases caused by numerous micro-organisms such as inflammatory bowel disease, Crohn's disease, urinary tract infections caused by E. coli and fatty liver abscess caused by K. pneumonia. The anti-microbial property was examined by extracting RS roots in different components such as aqueous, methanol, ethyl acetate, Chloroform and hexane, and testing them on selected micro-organisms. Aqueous extract of 100mg/ml concentration showed an inhibition of 16 (mm) for E. coli; whereas ethanol extract of 100mg/ml

concentration showed 2.4 (mm) for E. coli growth. This review presents the anti-microbial activity of different extracts of RS roots on different micro-organisms.

Keywords:

Apocynaceae, Gram-positive, Ayurveda, Root extracts.

14.1 Introduction:

Rauwolfia serpentina is also known as Sarpagandha it is an integral part of Ayurvedic medical system in India for over centuries for the treatment of various ailments. The leaves and roots of Sarpagandha contain alkaloids which are secondary metabolites. Major [1]. Sarpagandha is a species of flowering plant in the family Apocynaceae. *Rauwolfia serpentina* grows in tropical and subtropical forests.

In India, this plant has been used for the treatment of snake bites, feverish illnesses, and insanity for about 3000 years [2]. The biological-therapeutic significance of this plant has stimulated intensive studies, and many pharmacologically important alkaloids, such as Reserpine with sedative and antihypertensive activities and ajmaline with antiarrhythmic activity. About 80 alkaloids are isolated from Rauwolfia species among them reserpine is most important principal active constituent. M-reserpine is most important principal active constituent. The drug Sarpagandha is cardiodepressant, hypnotic and sedative.

It is used in hypertension, insomnia, sexual aggression and vertigo. The drug is much used in schizophrenia and conditions involving influence of evil spirits (Bhutawadha) [3].

14.2 Morphology:

Table 14.1: Morphology of Sarpagandha [4]

Plant parts	Morphological characteristics
Bark	Soft tap root, tuberous root, pale brown Cork
Leaf	3-5 whorls, ovate
Flower	White, pink, violet, irregular corymbs cymes
Fruit	Single, drupe and shinning black
Pulp	Pink or violet colour.
Inflorescence	Red pedicles, calyx and white corolla

The 74 accepted plants under the genus Rauwolfia got distributed in the tropical climates of Asia, Africa, South America, and diverse oceanic Islands. In India, it is found in the foothills of the Himalayas at an elevation of 1000 m and above, deciduous moist forests of the Eastern and Western Ghats, near lower Gangetic regions, Andaman Island [5].

Rauwolfia is shrub and evergreen, perennial, glabrous and the maximum height of the plant is 60 cm. The roots are tuberous with pale brown cork.

The leaves are three whorls, elliptic to lanceolate or ovate, below pale green and bright green above and it is thin. Its flowers are in irregular corymbose cymes, white, often tied with violet. March to May mounts flowering time according to Indian conditions.

The fruits are drupe, single or didymous, shining black, the inflorescence with red pedicles and calyx and white corolla [6].

The over-exploitation for Pharmaceutical benefts, indiscriminate collection by locals, and difficulty in the cultivation of this plant pose threats to its Existence in the wild. It led the International Union for The Conservation of Nature and Natural Resources (IUCN) to place *R. serpentina* in the IUCN Red List [7].

The plant usually grows to a height between 60 and 90 cm and has pale green leaves that are 7 to 10 cm long and 3.5 to 5.0 cm wide. The leaves are elliptical or lanceolate shaped and occur in whorls of 3 to 5 leaves.

The plant has many shiny, black or purple, round fruits that are approximately 0.5 cm in diameter. It also has small pink or white flowers. The plant has a prominent tuberous, soft taproot that reaches a length between 30 and 50 cm and a diameter between 1.2 and 2.5 cm [8].

Figure 14.1: Seed, Fruit, leaf, Root

14.3 Taxonomy:

Table 14.2: Taxonomy of Sarpagandha

Kingdom	Plantae
Phylum	Angiospermae
Subphylum	Eudicotidae
Class	Asterids
Order	Gentianales
Family	Apocynaceae
Genus	*Rauwolfia*
Species	*serpentina*

14.4 Vernacular Names:

Table 14.3: Vernacular names of Sarpagandha [9]

Language	Name
English	Serpentina root, Indian snakeroot, Rauvolfia root, serpentine root.
Hindi	Chotachand, Dhaval barua, Nayi, Rarnabheda, Herkaichandra, Chandrabhaga
Kannada	Sarpagandhi, Chandrika, sutranabhi, patalagaruda.
Tamil	Carpakanta, Savannamibori, Covannamilpori, Sarppaganti, Civanamalpodi.
Telugu	Patala Garuda, Patalagani, Patalagaruda, patalagandhi,

14.5 Phytochemical Constituents:

Rauwolfia serpentina has been a prevailing field of research for decades and several workers have explored this area due to its phytochemical properties. The various phytochemical compounds present in *sarpagandha* include alkaloids, phenols, tannins and flavonoids [10].

Reserpine: This is a pure single alkaloid extracted from the root of Sarpagandha in 1952. It is the most prominent of all the alkaloids and is used mainly as a natural tranquilizer. The antihypertensive properties of reserpine are due to its depressant action on the Central nervous system and peripheral nervous system. It prevents the normal storage of serotonin and catecholamine. It also interferes with functions of Autonomous nervous system by depleting catecholamine from the adrenergic neurons; it also activates the parasympathetic system. The overall effect is a reduction in blood pressure (antihypertensive properties), sedation and bradycardia [11].

Ajmalicine: Besides having blood pressure lowering properties it restores normal cerebral blood flow by its action on smooth muscles. It is estimated that about 3500 K.G. of Ajmelicine is isolated from Rauwolfia every year.

Phenols: Phenols are secondary plant metabolites. Their presence prevents the growth of pest and pathogens in the plant. It shows significant antidiabetic and hypolipidaemic properties.

Tannin and flavonoids: Tannins have astringent properties and they hasten the healing of wounds and control inflammation. Flavonoids are potent water-soluble antioxidants and free radical scavengers. Thus, they provide anti-inflammatory and anticancer activities. The Rauwolfia plant contains a large amount of macro and micronutrients. It is rich in calcium and zinc. It is a good source of ascorbic acid, riboflavin, thiamine, and niacin [12].

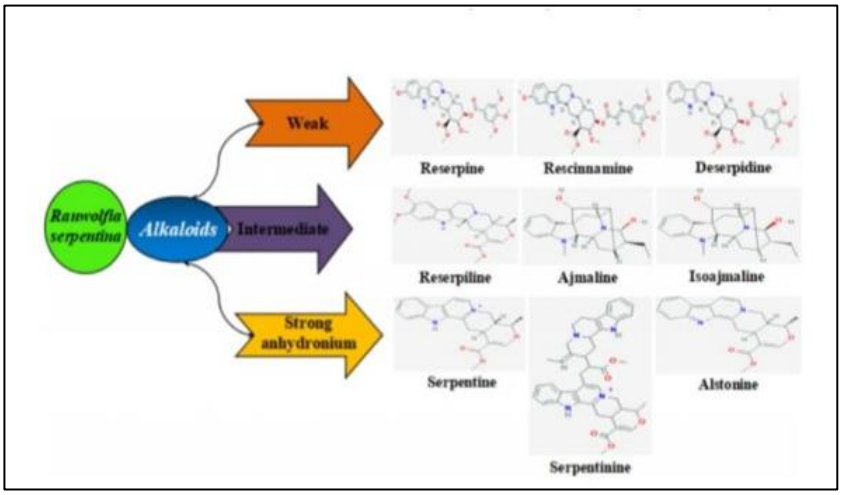

Figure 14.2: Chemical structure of phytochemicals of Sarpagandha [13]

14.6 Anti-Microbial Activity:

Sarpagandha is an important medicinal plant, mainly known for its various phytochemicals. Microbial pathogens develop resistance to antibiotics after repeated administration during the treatment of infectious diseases. It is felt necessary to find alternative antimicrobial drugs and the present trend is focused on medicinal plants [14].

14.6.1 Anti-Microbial Activity of Roots of Sarpagandha:

A. **Alka Rani Bunkar, 2007** studied anti-microbial activity of Sarpagandha on various micro-organisms such as *B. subtilis, K. pneumonia* and Staphylococcus species. Various extracts of Sarpagandha were treated to the bacterial culture, and zone of inhibition were measured. Methanolic extracts showed higher inhibition zone on *B. subtilis*, whereas Hexane and aqueous extracts showed no inhibition. Ethanolic extracts of Sarpagandha showed good inhibition on both *K. pneumonia* and staphylococcus

species [15].

B. **Aniel and his co-workers, 2016** carried research on anti-microbial activity of Sarpagandha, where different microorganisms such as bacteria, fungi and yeast were included. Hexane extract treatments of 100 mg/ml concentrations showed good inhibition in *E. faecalis, S. aureus and S. pneumonia*, but the same showed null inhibition in *P. aeruginosa and P. vulgaris*. Aqueous extracts showed greater inhibition in some of the bacteria such as *S. aureus, S. pneumonia* and *E. coli*. In case of fungal species such as

C. *albicans* and *A. niger*, chloroform and methanolic extracts of Sarpagandha exhibited higher inhibition whereas Aqueous and hexane showed no inhibition. The experiment was also carried out on *S. cerevesiae* species, where the zone of inhibition was higher when the cultures were treated with chloroform and methanolic extracts [16].

Table 14.4: Anti-microbial activity of roots of Sarpagandha

Sr. No.	Model	Treatment (ml)	Zone of Inhibition (mm)	Reference
01	*B. subtillis*	ETE (0.5) HE (100) CHLE (100) ME (100) AQEE (100)	10 - 17 24 -	Alka Rani Bunkar, 2017 Aniel *et al*.,2016
02	*Staphylococcus*	ETE 0.5	11	Alka Rani Bunkar, 2017
03	*K. pneumoniae*	ETE 0.5	15	Alka Rani Bunkar, 2017
04	*E. faecalis*	HE (100) CHLE (100) ME (100) AQEE (100)	16 22 20 -	Aniel *et al*.,2016
05	*S. aureus*	HE (100) CHLE (100) ME (100) AQE (100)	14 15 26 20	Aniel *et al*.,2016
06	*S. pneumoniae*	HE (100) CHLE (100) ME (100) AQE (100)	13 15 20 22	Aniel k et al.,2016
07	*E. coli*	HE (100) CHLE (100) ME (100) AQE (100)	10 14 14 16	Aniel *et al*.,2016
08	*P. aeruginosa*	HE (100) CHLE (100)	- -	Aniel *et al*.,2016

Sr. No.	Model	Treatment (ml)	Zone of Inhibition (mm)	Reference
		ME (100) AQE (100)	14 17	
09	*P. vulgaris*	HE (100) CHLE (100) ME (100) AQE (100)	- 14 15 -	Aniel *et al*.,2016
10	*C. albicans*	HE (100) CHLE (100) ME (100) AQE (100)	- 12 14 -	Aniel *et al*.,2016
11	*S. cerevisiae*	HE (100) CHLE (100) ME (100) AQE (100)	12 17 17 -	Aniel *et al*.,2016
12	*A. niger*	HE (100) CHLE (100) ME (100) AQE (100)	- 18 14 -	Aniel *et al*.,2016

(ETE – Ethanol extract, HE – Hexane extract, CHLE – Chloroform extract, ME – Methanol extract, AQE – Aqueous extract)

14.7 Conclusion:

The study suggested that methanol extract of Sarpagandha roots would be helpful in treating diseases caused by human pathogenic bacteria and fungi. In a particular, it can be recommended that the *R. serpentina* roots to be used for the control of infectious diseases caused by multidrug resistant *Staphylococcus aureus*.

It might turn out to be a good candidate in the search for effective antimicrobial agents**.** The results provide justification for the use of this plant in medicine to treat various infectious diseases. It is possible that better therapy for many microbial diseases can be found in the root extracts.

Preliminary results of this investigation indicate that Sarpagandha roots have high potential of antimicrobial activity. Also, antiproliferative activity of root and leaf extract of *R. serpentina* was analyzed which reported that among the two crude extracts, the leaf extract was found to be more effective for the antiproliferative activity. Thus, the presence of high level of indole alkaloids in the leaf and root extracts of Sarpagandha may be responsible for the observed potent antibacterial and antiproliferative activity of the samples. Therefore, this study reveals that the *R. serpentina* plant can be used as a natural therapy against bacterial infections as well as cervical cancer.

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15. Anti-Microbial Activity of Fenugreek (Trigonella Foenum-Graecum)

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Abstract:

Trigonella foenum-graecum also known as fenugreek is an annual plant belonging to the family Leguminosae. The green leaves and seeds of fenugreek are used in medicinal applications as well as in food.

Fenugreek is well known for its pharmacological properties including anti-bacterial, anti-oxidant, anti-cataract, hypocholesterolemia, anti-inflammatory and anti-diabetic activities. Methanol, chloroform, ethanol, hexane and acetone extract of fenugreek have potential antibacterial activity against gram positive and gram-negative bacteria such as E. coli, S. typhi, P. aeroginosa, B. marcescens, S. aureus, staphylococcus. Chloroform extract (20%) of fenugreek showed highest zone of inhibition of 38.4 mm in Shigella bacteria.

Aqueous extract of concentration (1000 mg/ml) showed minimum inhibition zone of 9.33 mm in P. aeroginosa. This review highlights antibacterial effect of fenugreek extracts that have been reported in several scientific studies.

Keywords:

Fenugreek, anti-microbial, zone of inhibition, Minimum inhibitory concentration (MIC).

15.1 Introduction:

Fenugreek is one of the major spices in the Indian diet. It provides natural dietary fiber and other important nutrients. Its leaves and seeds are used in foods and also in Ayurveda. Fenugreek contains a strong spicy and seasoning-type sweet flavor (1-2). Recently, there has been growing interest in the application of natural components as antimicrobial agents. Fenugreek seeds have shown potential as a dietary supplement (3).

The presence of galactomannan, the source of soluble dietary fiber in the endosperm of seed, improves the nutritional and physicochemical properties of the bread (4). In addition to being used in various food preparations, fenugreek also has healing benefits. The health promotion and disease prevention attributes of fenugreek are due to presence of diverse array of phytochemicals and their varying pharmacological and biological activities (5-6).

15.2 Morphology:

Fenugreek is a plant that stands around 2-3 feet tall where stems are erect and branched sometimes. It has green trifoliate leaves and its flowers are papilionaceous with lemon yellow color. Fruits occur as straight or sickle-like pods that contain solid rhomboidal, hard pebble-like shape seeds. Seeds are yellowish brown and light brown in color (7).

15.3 Nutritional Composition of Fenugreek Seed:

Fenugreek is one of the most ancient medicinal herbs. Fenugreek seeds contain moisture (8.84 g), ash (3.4 g), calories (323 kcal), carbohydrates (58 g), dietary fiber (25 g), protein (23 g), total fat (6 g), saturated fat (1.5 g), sodium (67 mg), potassium (770 mg) per 100 grams. Also contains β-carotene, ascorbate, iron, calcium, and zinc even more than regular food items (8).

15.4 Phytochemistry:

15.4.1 Alkaloids, Saponins, and Flavonoids in Fenugreek:

Different saponins, flavonoids, and alkaloids are present in fenugreek, but saponins are in higher concentration in fenugreek (9). Alkaloids and volatiles are the two major constituents of fenugreek seed which causes a bitter taste. These photochemical constituents act as antibacterial, hypoglycaemic, and cholagogic factors and their use promotes to manage lifestyle diseases (10).

15.5 Pharmacological Studies on *Trigonella Foenum-Graecum:*

Many bacterial Studies were conducted to demonstrate the antibacterial activity of fenugreek in gram positive and negative bacteria. This study suggests the potential of fenugreek seeds to become a source of antibacterial medication.

Dharajiya et al. performed an investigation to evaluate the antimicrobial potential of *Trigonella foenum-graecum* extracts and to elucidate the presence of phytochemicals responsible for its biological activity. The extracts were prepared by sequential cold maceration method by using solvents viz., hexane, ethyl acetate, and methanol. Antimicrobial activity of extracts was carried out by agar well diffusion method against four bacteria. Minimum inhibitory concentration (MIC) of different extracts was determined using the broth dilution method. Thin layer chromatography (TLC), TLC bioautography and phytochemicals analysis were also performed. The antibacterial activity found maximum against *S. marcescens* with a zone of inhibition (ZOI) of 12.33 mm by aqueous extract and minimum on *E. coli* with a zone of inhibition (ZOI) of 9.3 mm by methanol extract (11).

Alwan et al. conducted a study to evaluate the antibacterial activity of methanol and aqueous extract of fenugreek. The study detected antibacterial activity by using well diffusion in agar technique. The study applied on the species of bacteria includes, *Staphylococcus aureus, Bacillus subtilus, Pseudomonas aeruginosa* and *Escherichia coli.*

The study showed highest antibacterial activity of methanolic extract of seeds of *Trigonella foenum –graecum* that attained highest zone of inhibition (30 mm) against *P. aeruginosa*, while attained lowest zone of inhibition (17 mm) against *S. aures* using aqueous extract (12).

Sharma et al. conducted a study to investigate antibacterial activity of fenugreek extract (Methanol, Acetone and aqueous extract) against *E. coli* and *Staphylococcus* was determined by the well diffusion method. The maximum zone of inhibition was given by methanol i.e., 20 mm and 19 mm against *E. coli* and *Staphylococcus* respectively, followed by Acetone extract which give the equal zone of inhibition for both organisms i.e., 16 mm while the aqueous extract shows nil zone of inhibition. Thus, from bacteriological point of view fenugreek appears to play a great role in clinical as well as antibacterial agents (13).

Abdalah et al. conducted an antimicrobial study on the seeds extract of fenugreek, which is determined by using agar diffusion method to measure the antimicrobial activity on bacterial isolated from different sources such as *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeroginosa, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae.* The results of antimicrobial analysis identified that *E. coli* is the most sensitive pathogen to methanol extract of fenugreek seeds with maximum zone of inhibition (ZOI) of 26 mm (14).

Alwhibi et al., screened fenugreek for phytochemical active constituents and investigated for antimicrobial properties against a selection of gram- positive and gram-negative pathogenic bacteria. Five different solvent seed extracts were tested. The results of the study

demonstrated that the chloroform and methanolic extracts possessed significant antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi* and *Klebsiella pneumonia.* The antimicrobial activity of the extracts was investigated using the agar well diffusion method. The results of the antimicrobial analysis identified *Shigella sonnei* is the most sensitive pathogen to the chloroform extract of fenugreek seeds with maximum zone of inhibition (ZOI) of 38.4 mm (15).

Table 15.1: Antimicrobial Activity of Fenugreek (ME- Methanol extract, EE- Ethanol extract, AqE- Aqueous extract, CE- Chloroform extract, HE- Hexane extract, AE- Acetone extract)

Sr. No.	Model	Treatment	Result (Zone of inhibition in mm)	Reference
1.	*E. coli*	ME [100 mg/ml] EE [100 mg/ml] AqE [100 mg/ml] HE [100 mg/ml]	9.34 - 10.50 -	11
	P. aeroginosa	ME [100 mg/ml] EE [100 mg/ml] AqE [100 mg/ml] HE [100 mg/ml]	9.50 - 9.33 -	11
	B. cereus	ME [100 mg/ml] EE [100 mg/ml] AqE [100 mg/ml] HE [100 mg/ml]	11.50 11.33 10.33 -	11
	S. marcescens	ME [100 mg/ml] EE [100 mg/ml] AqE [100 mg/ml] HE [100 mg/ml]	10.00 - 12.33 9.66	11
2.	*E. coli*	ME [75%] AqE [75%]	20 22	12
	B. subtilus	ME [75%] AqE [75%]	25 20	12
	P. areuginosa	ME [75%] AqE [75%]	30 24	12
	S. aureus	ME [75%] AqE [75%]	20 17	12
3.	*E. coli*	ME [75%] AqE [75%] AE [75%]	20 - 16	13
	Staphylococcus	ME [75%] AqE [75%] AE [75%]	19 16 -	13

Sr. No.	Model	Treatment	Result (Zone of inhibition in mm)	Reference
4.	*S. aureus*	ME [1000 mg/ml] AqE [1000 mg/ml]	24 -	14
	P. aeuroginosa	ME [1000 mg/ml] AqE [1000 mg/ml]	20 -	14
	E. coli	ME [1000 mg/ml] AqE [1000 mg/ml]	26 -	14
	Proteus vulgaris	ME [1000 mg/ml] AqE [1000 mg/ml]	17 12	14
	Klebsiella pneumonia	ME [1000 mg/ml] AqE [1000 mg/ml	24 -	14
	Streptococcus pyogenes	ME [1000 mg/ml] AqE [1000 mg/ml]	17 19	14
5.	*E. coli*	AqE [20%] CE [20%] ME [20%] EE [20%]	18.2 28.2 25.2 22.6	15
	P. aeuroginosa	AqE [20%] CE [20%] ME [20%] EE [20%]	22.3 35.6 29.7 28.4	15
	S. typhi	AqE [20%] CE [20%] ME [20%] EE [20%]	22.4 34.6 28.8 -	15
	S. auerus	AqE [20%] CE [20%] ME [20%] EE [20%]	18.7 27.4 30.3 16.2	15
	Klebsiella pneumonia	AqE [20%] CE [20%] ME [20%] EE [20%]	25.7 36.3 25.5 11.1	15

Sr. No.	Model	Treatment	Result (Zone of inhibition in mm)	Reference
	Shigella sonnei	AqE [20%] CE [20%] ME [20%] EE [20%]	- 38.4 27.3 19.1	15

15.6 Conclusion:

Several health-beneficial attributes of the fenugreek, have been experimentally evidenced in recent years, which have the potential for possible therapeutic application. Fenugreek is rich with a wide variety of metabolites such as tannins, alkaloids, flavonoids, terpenoids and glycosides which are known to have antimicrobial properties.

Chloroform extract (20%) of fenugreek showed highest zone of inhibition of 38.4 mm in *Shigella* bacteria, whereas aqueous extract of fenugreek showed lowest zone of inhibition of 9.33 mm in *P. aeroginosa*. Thus, from bacteriological point of view fenugreek appears to play a great role as antibacterial agents.

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