Volume I

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

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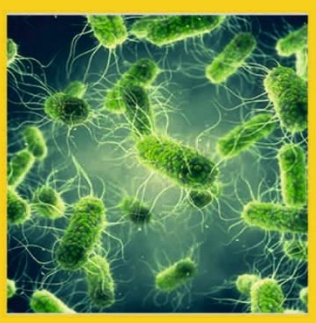
Dr. Talluri Rameshwari K. R.

Dr. Kshitij Singh

Dr. Ajay Kumar Singh



Microbiological Association for Science and Technology Development (MASTD) Lucknow Uttar Pradesh, India.

Genespy Research Services Pvt. Ltd. Mysuru, Karnataka, India.

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

(Volume I)

Editors

Dr. Talluri Rameshwari K. R.

Research Associate, Department of Microbiology,
JSS Academy of Higher Education & Research,
Mysuru, Karnataka.

Dr. Kshitij Singh

Department of Environmental Science,
Babasaheb Bhimrao Ambedkar University (A Central University),
Lucknow, Uttar Pradesh.

Dr. Ajay Kumar Singh

Ministry of Environment Forest and Climate Change Department,
Uttar Pradesh, India.

Microbiological Association for Science and Technology Development (MASTD) Lucknow Uttar Pradesh, India.

Genespy Research Services Pvt. Ltd. Mysuru, Karnataka, India.

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Editors By: **Dr. Talluri Rameshwari K. R., Dr. Kshitij Singh, Dr. Ajay Kumar Singh**

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Sus Road, Pashan- 411021, Pune, Maharashtra, India.
Mob: +91-8007068686
Email: editor@kdpublications.in
Web: https://www.kdpublications.in

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. A Novel Approach on Colchicine Microsponge-Based Gel for Topical Drug Delivery System: Updated Review

Lalita, Twinkle Garg, Gaurav Kumar

Himalayan Institute of Pharmacy Kala-Amb,
Teh. Nahan, Sirmour (H.P.).

Abstract:

Topical administration is the most preferred method for delivering medicinal substances locally because it is quick and inexpensive. Getting the best possible concentration of a specific medicine at the site of action for the right amount of time is the specific problem of creating a therapeutic system. In topical medication delivery, the drug is transferred from a skin-applied substance to a local target location before being eliminated from the body and deeper tissues through diffusion, metabolism, and dermal circulation. A polymeric delivery device made of porous microspheres is known as a microsponge. They are little spheres that resemble sponges and have thick layers of pores on their surface. They could also improve stability, lessen side effects, and favorably alter medication release. The numerous positive aspects of microsponge technology make it a flexible means of drug administration. In recent years, oral medication administration has begun to use microsponges, typically used in topical drug delivery. Microsponges respond to external stimuli and release their medicine at regular intervals. Gout, pseudo-gout, and familial Mediterranean fever, among many other ailments, are all treated with colchicine. Alkaloid colchicine transdermal gel is used to treat and prevent gout flare-ups in adults. Poor bioavailability and severe gastrointestinal side effects are linked to oral administration. It is also well known that the medicine has a low therapeutic index. Therefore, the topical administration of colchicine was examined using microsponge-based gels as possible carriers to address these limitations.

Keywords:

Colchicine, microsponge, topical administration, alkaloid.

1.1 Introduction:

The route of a particular drug carrier system is always a crucial platform of development that combines the concepts of biomedical technology, nanotechnology, and pharmaceutical drug design. Throughout the past few decades, interesting developments in route-specific drug carrier systems have resulted from advances in material sciences. The most common drug administration methods are oral, intramuscular (IM), intravenous (IV), subcutaneous (SC), and transdermal (TD). The TD system includes the diffusion of medicines over the epidermal layer into a prolonged release after passing through the skin's stratum corneum (1-3). Because of its relatively straightforward and non-invasive administration, this

technique, also known as topical medication delivery, has noticeably increased study interest in recent years. The main area of concern is the enhancement in drug bioavailability via the TD route, which can lessen the effects of first-pass metabolism encountered by the oral route. Another element that makes TD drug delivery more appealing is the reduction of the negative effects associated with other drug administration routes. Drugs like ranitidine or oestradiol, for example, can be successfully supplied by the TD route, which can resolve the harmful characteristics of an oral route, such as limited bioavailability and liver damage, respectively (4-6).

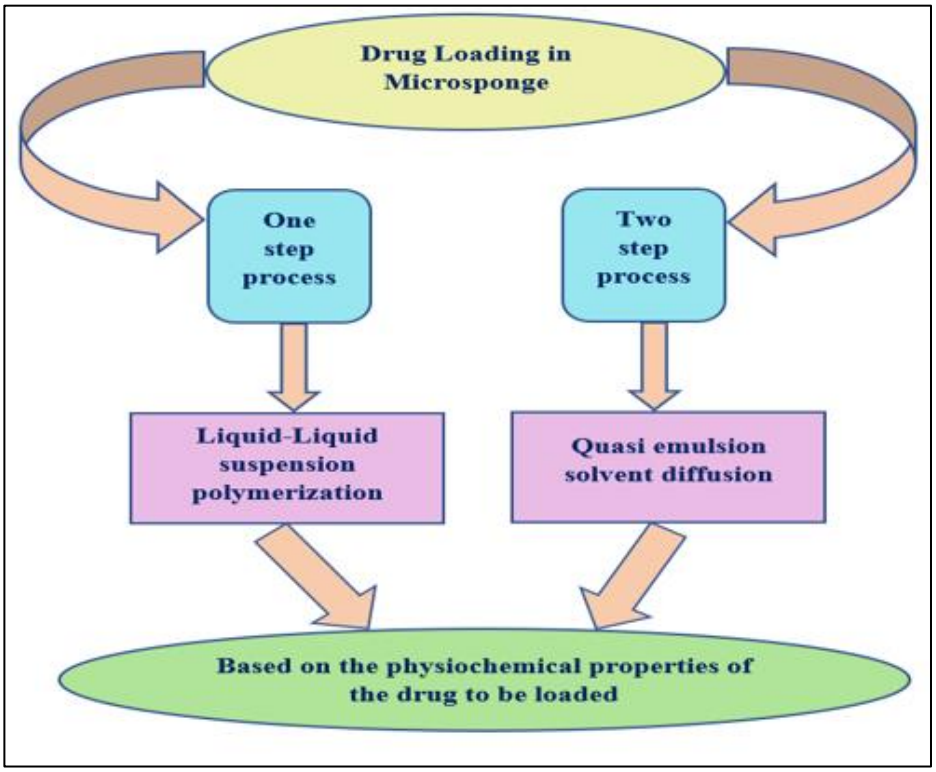

Figure 1.1: Preparation Method of Microsponges

The creation of innovative microsponge-based drug delivery systems has received a lot of attention in recent years to alter and regulate the release behavior of pharmaceuticals, and as the preparation method of microsponges is shown above in the figure 1. Changing the therapeutic index and duration of a drug's activity is possible by putting it into a carrier system. The extensive use of substances like -hydroxy acids and vitamins in topical solutions, which can induce perceivable and observable effects - notably in aging or photodamaged skin - has encouraged consumers' growing interest in skincare and skin treatment products. Despite being very helpful, these substances can occasionally cause irritation, which is often felt as burning, stinging, or redness and is more common in people with sensitive skin. After realizing the issue, the formulators attempted to address this issue using one of the two techniques. They sacrificed efficacy to lower the concentration of these substances. Also, the vehicle has been altered to improve the product's skin compatibility or emollience.

The healthcare system has been greatly impacted by medication delivery devices that can precisely manage the release rates or target pharmaceuticals to a specific bodily spot. Transdermal delivery systems (TDS), which use the skin as an entrance point, are a group of predictable and dependable methods that have been created for systemic medications. Several medications that could be administered more effectively through the skin have seen an improvement in efficacy and safety. Nevertheless, compounds intended for the skin cannot be transported by TDS (7-9). Using topical medications also has several drawbacks, including frequent unsightly, greasy, sticky ointments, and other issues that frequently lead to poor patient compliance. Due to their ineffective delivery systems, these vehicles need a large concentration of active ingredients to be therapeutically effective, which can irritate and induce allergic reactions in some users. Additional downsides of topical preparations include uncontrolled evaporation of the active ingredient, offensive odor, and possible drug-vehicle incompatibility (10).

Microsponges are minute spheres that can absorb skin secretions, lowering the skin's oiliness and gloss. Skin secretions can be held in spheres that are made up of clusters of even smaller spheres and can hold four times their weight. Very tiny, inert, unbreakable spheres known as microsponge particles do not penetrate the skin. Instead, they gather in the minuscule crevices of the skin and gradually release the medicine as the skin requires it. The microsponge system can avoid an excessive build-up of components in the dermis and epidermis. Microsponges are proprietary polymeric delivery systems made of porous microspheres that can hold a variety of active substances, including sunscreen, emollients, perfumes, essential oils, and anti-infective, anti-fungal, and anti-inflammatory medications (11).

A. Many Scientists Gave Their Reviews About the Colchicine Microsponge-Based Gel for Topical Drug Delivery Systems by Using Different Methods and Drugs:

VS Sivasankarapillai *et al.*, (2021) reported that the path of a particular drug carrier system is always an important development platform that integrates the concepts of biomedical technology, nanotechnology, and pharmaceutical drug design. Transdermal (TD) drug delivery includes the drug being released from the stratum corneum of the tissue membrane into a sustained release by diffusion over the epidermal layer. Because of its comparatively straightforward and non-invasive administration, this approach (commonly referred to as topical medication delivery) has noticeably increased study interest in recent years. Over the past several decades, significant progress was made in TD delivery, and a few medications are currently successfully reported. In this study, we emphasize developments in the use of significant biopolymers mentioned for TD drug release applications and related areas. For the creation of the TD carrier system, three commonly reported plant- and animal-derived polymers (such as natural rubber, chitosan, and cellulose) were thoroughly examined (12).

H Shang *et al.*, (2022) reported that Accidents, sports, aging, diseases, and other factors can all cause arthritic injuries over the course of a lifetime. These injuries can include synovitis, inflammation, discomfort, tendon and ligament damage, cartilage/bone damage, and tendon and ligament damage.

Therapeutic drugs are often administered orally or intravenously, although both methods have numerous negative effects. A different method for effective and safe delivery is transdermal application. Non-steroidal anti-inflammatory drugs have been available in transdermal formulations for many years and exhibit promising efficacy in the treatment of pain, inflammation, infection, and other conditions. Novel transdermal patches, gels/films, and microneedles have also received a lot of attention as delivery systems for therapies intended to treat arthritic injuries. Transdermal formulations that stop disease development and encourage damage repair, however, take time to get from the lab bench to clinical applications. The effectiveness of transdermal administration is constrained in part by the skin barrier and synovial capsule barrier. Several nanocarriers, including nanoparticles, nano lipids, nanoemulsions, nanocrystals, exosomes, etc., have recently been added to transdermal formulations to improve drug delivery. The combined transdermal formulations exhibit encouraging safety and effectiveness. The current state of transdermal formulations based on nanomedicine for the treatment of arthritic injuries will therefore be the main emphasis of this review. To drive further research and speed up clinical translational investigations, the developments, restrictions, and outlooks in this field will also be examined (5).

De Oliveira BE *et al.*, (2021) reported that Using nanotechnology to deliver 5-FU has demonstrated good results in treating premalignant and malignant skin disorders, even in deeper layers of the skin. This could further advance the treatment of various types of skin cancer and lower the mortality rate. We compiled most papers on novel medication delivery systems for 5-FU. To incorporate some of these technologies into routine clinical practice, more study is required, including randomized and controlled clinical studies. The approval and production of commercial topical treatments will also require the interest of pharmaceutical corporations (13).

AbouSamra MM *et al.*, (2019) reported that the goal of this study was to create and assess nystatin (Nys) nanocapsular hydrogel as a desirable dosage form for the management of topical candidiasis. Using the nanoprecipitation technique, nanocapsular dispersions were created. The impact of formulation components, specifically the concentrations of polycaprolactone (PCL) (0.25, o.5% w/w), squalene (2.5, 5% w/w), and span 60 (Sp60) (0.5, 1% w/w), on the various physicochemical features of the formulations created, was examined. The outcomes demonstrated the significance of the investigated parameters, which were optimized, and the nanocapsular formulation; Nys-NC6, which is made up of 0.25% weight-per-weight PCL, 5% weight-per-weight squalene, and 1% weight-per-weight Sp60, demonstrated the most beneficial characteristics. High encapsulation effectiveness (86.09 0.28%), a small mean diameter (254 6.81 nm), a low polydispersity index (0.23 3.03), and a strong negative zeta potential (48 2.00 mV) were all characteristics of Nys-NC6.The in vivo antifungal activity of Nys-NC6 hydrogel was investigated against Candida albicans utilizing an infected rat model after incorporation into Carbopol gel. By significantly reducing the fungal count and eliminating the Candida infection, Nys-NC6 hydrogel demonstrated a superior antifungal effect in comparison to Nys hydrogel and the market product after the treatment period. Confocal microscopy and histopathology photomicrographs, which confirmed the tested formulation's antifungal activity, provided additional support for the conclusions. According to the latest research, topical candidiasis can be successfully treated with nystatin nanocapsular hydrogel (14).

Karn AK *et al.*, (2021) suggested that Nutraceuticals, which refers to "food" with medicinal characteristics, are a mix of nutrition and pharmaceuticals. Nutraceuticals are divided into conventional (herbs, phytochemicals, probiotics, and prebiotics) and non-traditional (fortified and recombinant) categories; they are mostly used for illness prevention and treatment. Nutraceuticals have several therapeutic properties that can be used to treat diseases like Alzheimer's, Parkinson's, diabetes, cancer, and cardiovascular conditions. Because they are mostly made from natural sources, nutraceuticals are safer and more affordable than medicines, which has resulted in explosive market expansion for this category of goods. Determining the progress made in the field of nutraceuticals and their potential as pharmaceutical substitutes is the main goal of this review. The advantages that nutraceuticals have over pharmaceutical products are the main topics of this essay, which also examines the nutraceuticals' historical and current market situations. Second, the shortcomings of nutraceuticals have been addressed and their efficacy has been increased thanks to the development of novel drug delivery systems. This has sparked the interest of scientists and researchers in this field for further advancement. As a result, nutraceuticals can be expected to have a prosperous market in the coming years. Nutraceuticals have a significant potential to substitute medications and benefit human health (15).

Mavuso S. *et al.*, (2016) suggested that Genetic auto-inflammatory inflammatory skin disorders (GAISDs) are a class of genetic diseases that are characterized by repeated bouts of severe localized inflammation and fever that appear to be unrelated to any external cause. The innate immune system is abnormally activated in GAISDs, which results in clinical inflammation and elevated levels of acute-phase reactants. To administer [Copper-glycylglycine-prednisolone succinate] ([Cu(glygly)(PS)]) with dual pH/redox responsiveness, a phospholipid-based system with Eudragit® E100 (EuE100) chemically modified into EuE100-cystamine derivative was created in this study. Comparing the biological actions of these two substances helped to explain why [Cu(glygly)(PS)] complex should be used instead of pure PS corticosteroid. In comparison to the free PS medication, the results showed that [Cu(glygly)(PS)] had a significant inflammatory/oxidant inhibitory activity. In contrast to PS, which provided the activity of 4.4% and inhibition of 6.1% and 2.6%, respectively, the [Cu(glygly)(PS)] complex showed a considerable free radical-scavenging activity (60.11.2%) and lipoxygenase (LOX-5) inhibitory activity (36.61.3%). The [Cu(glygly)(PS)] loaded NLs demonstrated a low level of [Cu(glygly)(PS)] release of 22.95.4% in 6h at pH 7.4, as opposed to an accelerated release of 75.93.7% in 6h at pH 5 in a reducing environment. After that, [Cu(glygly)(PS)]-loaded nano liposomal gel, also known as dermal sludge, was created by dispersing optimum [Cu(glygly)(PS)]-loaded NLs in hydroxypropyl methylcellulose (HPMC)/polyvinyl alcohol (PVA) gel. A viscous gel suspended with solid particles ([Cu(glygly)(PS)]-loaded nanoliposomes) is referred to as cutaneous sludge. Ex vivo permeability, in vitro release, cytotoxicity, and in vivo tests were used to describe the sludge and compare it to traditional PS formulations. According to the results, the unique dual redox/pH responsive nano liposomal dermal sludge has a great deal of potential for targeted bioactive delivery in TRAPS via the transdermal route, therefore increasing the therapeutic effect (16).

Atanasova D. *et al.*, (2021) reported that the medicine used for topical and transdermal skin treatments in medical and clinical settings has been examined in this review. This includes a description of the nature of human skin, the method for transdermal BAS distribution, and the variables affecting delivery effectiveness. It has also been demonstrated that BAS can

be applied directly to the textile matrix, but that encapsulation or bonding to an appropriate carrier will allow for distribution management. Several stimuli-responsive drug carriers, including dendrimers, micelles, liposomes, nanoparticles, and hydrogels, are promising, and have undergone extensive research. Textile-based bioactive composites offer a fresh perspective on biomaterials for the creation of medical devices including patches, bandages for wounds, BAS carriers for transdermal therapy, carriers for degenerative disorders, etc. In a particular context, the typical textile qualities combined with controlled BAS administration and the ability to visually track the drug delivery process will offer information, convenience, and prompt action. The health and social standing of people will unquestionably improve as a result (17).

B. Advanced Technologies Used in Colchicine Microsponge-Based Gel for Topical Drug Delivery System:

A. **Recent advances on transdermal delivery systems for the treatment of arthritic injuries:** From classical treatment to nanomedicines: Over a person's lifetime, arthritic injuries are common owing to accidents, sports, age, diseases, etc. These injuries can cause synovitis, inflammation, pain, and/or damage to the cartilage or bone and the tendon or ligament. Therapeutics are often administered orally or intravenously, although both methods have a few negative side effects. Another option for effective and safe delivery is transdermal administration. Non-steroidal anti-inflammatory drug transdermal formulations have been on the market for years and exhibit potential efficacy in treating pain, reducing inflammation, preventing infections, and other conditions. Novel transdermal patches, gels/films, and microneedles have also been extensively investigated as delivery systems for medicines to treat arthritic wounds. Nevertheless, the transition from the lab bench to clinical applications for transdermal formulations that stop disease development and encourage damage repair is slow. The synovial capsule and skin barriers, which restrict the efficacy of transdermal administration, are significant factors. To improve medication delivery, numerous nanocarriers, including nanoparticles, nano lipids, nanoemulsions, nanocrystals, and exosomes, have recently been added to transdermal formulations. The combined transdermal formulations exhibit encouraging levels of safety and effectiveness. Consequently, the focus of this study will be on outlining the current state of transdermal formulations based on nanomedicine for the treatment of arthritic injuries. To foster future research and quicken clinical translational investigations, the advancements, limitations, and prospects in this sector will also be discussed (12).

B. **Progress in natural polymer engineered biomaterials for transdermal drug delivery systems:** The development of a particular drug carrier system's route is always a key platform that blends the concepts of biomedical technology, nanotechnology, and pharmaceutical drug design. Drugs are released via the stratum corneum of the tissue membrane during transdermal (TD) drug delivery, allowing for a prolonged release by diffusion across the epidermal layer. Due to the comparatively straightforward and non-invasive administration of this approach, which is sometimes referred to as topical drug delivery, research interest has expanded noticeably over the past few decades. Several medications are now successfully reported thanks to the significant advancements made in TD delivery over the past few decades. In this study, we concentrate on the advancements made in the applications of significant biopolymers that were previously discussed for TD drug release applications and related areas. Three commonly

mentioned plant- and animal-derived polymers (such as chitosan, cellulose, and natural rubber for the development of TD carrier system) were thoroughly examined. The overall principle of TD medication delivery was covered as well as discussing the benefits and shortcomings of the reported experiments (5).

C. **5-Fluorouracil, innovative drug delivery systems to enhance bioavailability for topical use:** In this article, most of the research on novel drug delivery systems for 5-FU is summarised. Some of these technologies need to be incorporated into routine clinical practice, which calls for additional studies, such as randomized and controlled clinical trials. The approval and production of commercial topical treatments will also require the interest of pharmaceutical corporations. Even in deeper layers of the skin, 5-FU delivery by nanotechnology has demonstrated encouraging results in the treatment of premalignant and malignant skin illnesses, which may further advance the management of several skin cancer types and lower the mortality rate (13).

D. **A promising nystatin nanocapsular hydrogel as an antifungal polymeric carrier for the treatment of topical candidiasis:** The creation and assessment of nystatin (Nys) nanocapsular hydrogel as a desirable dosage form for the treatment of topical candidiasis was the goal of the current work. The nanoprecipitation technique was used to create the nanocapsular dispersions. On the various physicochemical features of the formulations developed, the effects of formulation elements such as polycaprolactone (PCL) concentration (0.25, o.5% w/w), squalene (2.5, 5% w/w), and span 60 (Sp60) (0.5, 1% w/w) were examined. The outcomes demonstrated the importance of the components that were investigated and optimized, and the nanocapsular formulation, Nys-NC6, which included 0.25% w/w PCL, 5% w/w squalene, and 1% w/w Sp60, demonstrated the most advantageous characteristics. Nys-NC6 had a high encapsulation efficiency of 86.09 0.28%, a small mean diameter of 254 6.81 nm, a low polydispersity index of 0.23 3.03, and a high negative zeta potential of 48 2.00 mV. Nys-NC6 hydrogel's in vivo antifungal activity was examined against Candida albicans utilizing an infected rat model after being included in Carbopol gel. By significantly reducing the fungus count and eliminating the Candida infection after the treatment period, Nys-NC6 hydrogel outperformed Nys hydrogel and the market product in terms of antifungal effectiveness. Confocal microscopy and histopathology photomicrographs that confirmed the antifungal activity of the tested mixture provided additional support for the results. The results of the current study revealed that nystatin nanocapsular hydrogel is a useful method for treating skin candidiasis (14).

- Viability of Nanostructured Lipid Carrier System in Overcoming the Barriers Associated with Chemotherapeutic Delivery: Due to some inherent issues with anti-cancer agents, including instability, low solubility, non-specificity, variable pharmacokinetics, a small therapeutic window, the development of multi-drug resistance, and other physiological barriers related to tumour cells, delivery of these agents is difficult. Nanostructured lipid carriers (NLC) have grown significantly in significance in recent years for enhancing the therapeutic efficacy of anti-cancer medicines. The current review provides a thorough explanation of the many obstacles to delivering the anti-cancer agent, the suitability of NLC to deliver the anti-cancer agent, the methods used to create NLC, its structure, and its characterization. Several qualitative pieces of literature have been included in this evaluation because the main emphasis has been on finding ways to get beyond current obstacles in the delivery of chemotherapeutics by NLC. The report also goes into detail about the stability problem related to NLC's long-term storage. The NLCs systems have a significant deal of

promise to target different anti-cancer medicines that have poor solubility, lack of specificity, and severe side effects. The advancement of the NLC system can remove obstacles in the delivery of anti-cancer medications and enhance its effectiveness in treating different types of melanomas (18).

- Textile Materials Modified with Stimuli-Responsive Drug Carrier for Skin Topical and Transdermal Delivery: In skin topical or transdermal therapy, textile materials can play a significant role as a suitable matrix for various active compounds that facilitates their progressive release. These materials are distinguished by their compositional and structural diversity and easily satisfy the requirements for use in particular therapies. Textiles can be employed as carriers in a variety of medical procedures, including aromatherapy, the delivery of antibiotics and painkillers, hormone therapy, and the treatment of psoriasis, atopic dermatitis, and melanoma. Biologically active compounds can be loaded onto textile materials using a variety of optional techniques. The oldest ones use the pad-dry-cure process, spraying, and exhaustion. Microencapsulation is a common technique. To create new textiles with enhanced multifunctional capabilities and intelligent responsiveness, stimuli-responsive polymers can be added to textile materials (17).

C. Scope for Colchicine Microsponge-Based Gel for Topical Drug Delivery System:

Microsponges are porous, polymeric microspheres that have lately been used for oral delivery but are primarily employed topically. Microsponges are created to effectively administer a pharmaceutically active component at the lowest amount possible, as well as to improve stability, lessen adverse effects, and alter drug release (19).

The epidermis and dermis of people who use Microsponges Drug Delivery System do not accumulate the active component. By maintaining their efficacy, Microsponge Drug Delivery System lessens irritation from medications that work. Patient adherence to Microsponge Drug Delivery System is present.

On the skin's surface or in the epidermis, Microsponge Drug Delivery System lengthens a drug's residence period. A micro sponge's benefits include the potential to alter drug release, improve drug stability, and significantly lessen side effects (20).

The Microsponge Delivery System (MDS) uses macroporous beads that are typically 10–25 nm in diameter and filled with the active ingredient to deliver topical medicines in a regulated manner. Polymeric delivery devices called microsponges are made of porous microspheres. These are small, spherical particles that resemble sponges and have a highly porous surface.

In addition, they could improve stability, lessen side effects, and improve medication release. In general, there are two ways to make microsponges: liquid-liquid suspension polymerization and quasi-emulsion solvent diffusion. However, new techniques have recently been discovered, and it is important to consider both the benefits and drawbacks of these approaches. (22,23) Examples of Polymers used in microsponges gel formulations are listed below in table 1.1.

Table 1.1: Examples of Polymers Used in Microsponges Gel Formulations.

Sr. No	Polymers
1	Eudragit RS100
2	Carbopol 934
3	Polymethacrylates
4	Ethyl cellulose
5	Polylactic acid
6	Polyhydroxy butyrate

Microsponges are small spheres that can absorb skin secretions to lessen skin shine and oiliness. Microsponges are porous microsphere-based polymeric delivery devices. These are tiny, spherical particles with porous surfaces that resemble sponges.

Moreover, they might improve stability, lessen side effects, and favorably alter medication release. Microsponges can be used in formulations including creams, powders, gels, and lotions to entrap different kinds of medications (23)., A microsponges delivery system fixes the drawbacks of topical preparation, such as their unpleasant Odour, greasiness, skin irritability, and inability to reach the systemic circulation.

By trapping less soluble drugs inside their pores, microsponge systems improve the solubilization of these drugs.

The medicine is successfully reduced to minute particles due to these tiny holes, and increasing the surface area will speed up the solubilization process (24). The examples of drugs used as microsponges based gel formulations against some diseases are discussed below in table 1.2.

Table 1.2: Examples of drugs used as microsponges based gel formulations against some diseases. (25)

Drugs	Diseases
Terbinafine HCl	Anti-fungal
Hydroxyzine HCl	Urticaria and atopic dermatitis
Mupirocin	Antibacterial activity
Fluconazole	Inflammatory diseases
Benzoyl peroxide	Anti-Acne Treatment
Acyclovir	Viral infections

1.2 Applications of Microsponges Gel Formulations:

Microsponges are considered to effectively deliver a pharmaceutical active ingredient at the lowest dose while also improving stability, reducing adverse effects, and altering drug release. (26) The layout of microsponges applications is shown below in figure 2, while applications of microsponges gel formulations are also discussed below in table 1.3.

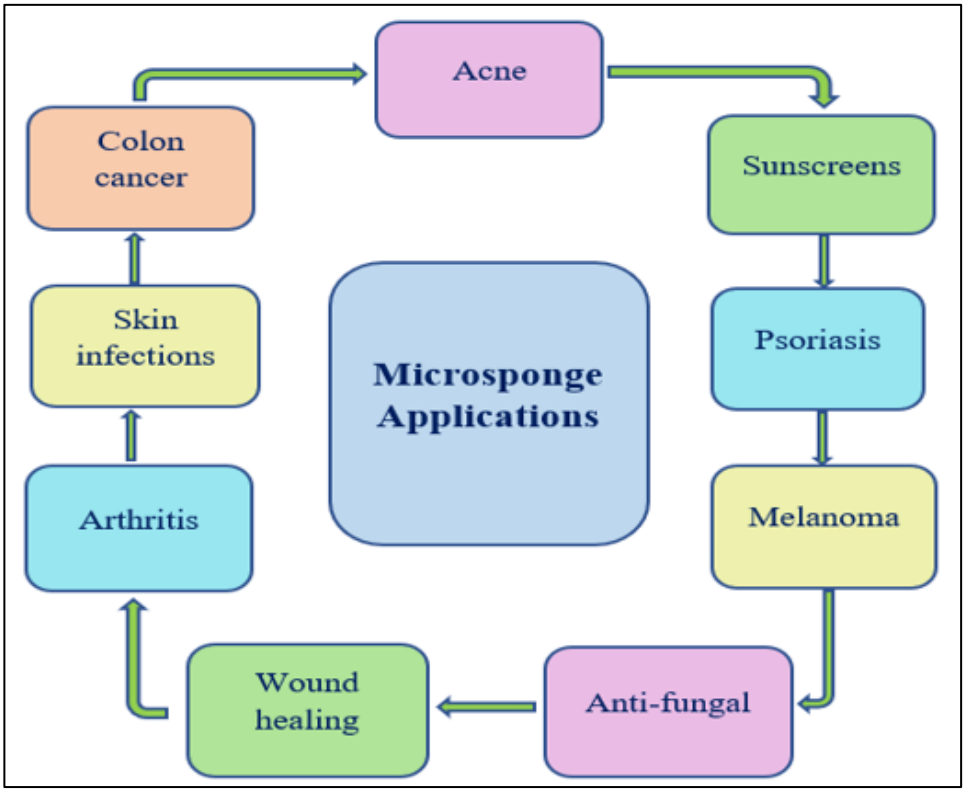

Figure 1.2: Microsponge Applications

Table: 1.3 Applications of Microsponges Gel Formulations

Sr. No	Formulations	Active component	Applications	References
1	Dermatitis	Miconazole	Diaper dermatitis can be treated with microsponge gel for improved therapeutic results.	27
2	Antifungal	Bifonazole	Using the aqueous emulsion diffusion evaporation method, microsponges gel increased patient compliance.	28

Sr. No	Formulations	Active component	Applications	References
3	Arthritis therapy	Diclofenac Diethylamine	Diethylamine microsponge gel with extended release provides effective arthritis treatment.	29
4	Anti-inflammatory	Betamethasone	Topical gel made from betamethasone microsponges using the Quassi emulsion solvent diffusion process.	30
5	Anti-Ulcer	Femotidine	Microsponges provide controlled release by Quassi emulsion solvent diffusion method.	31
6	Antifungal infection	Fluconazole	Microsponge gel for antifungal infection treatment.	32
7	Schizophrenia	Risperidone	For controlled release by Quassi emulsion solvent diffusion method	33
8	Antifungal	Sertaconazole nitrate	Topical formulation with microsponges that delivers the drug in a controlled manner.	34

1.3 Conclusion:

Transdermal delivery systems (TDS), which rely on the skin as an entry route, are a collection of trustworthy and predictable techniques developed for systemic drugs. Improvements in efficacy and safety have been made for some drugs that could be delivered more successfully through the skin. TDS cannot convey substances meant for the skin, though. The use of topical drugs is not without its downsides, which typically include ointments that are unattractive, greasy, and sticky as well as other problems that frequently result in low patient compliance. Owing to their inefficient delivery systems, these medications require a high concentration of active components to be therapeutically effective, which can irritate some users and cause allergic responses. Topical formulations also include drawbacks such as uncontrolled evaporation of the active ingredient, unpleasant odour, and potential drug-vehicle incompatibility. Little spheres called microsponges can take in skin secretions, reducing the skin's oiliness and gloss. Spheres composed of groups of even smaller spheres can hold four times their weight in skin secretions and can be used to store them. The skin is not penetrated by the incredibly small, inert, indestructible spheres known as microsponge particles. Instead, they assemble in the skin's microscopic cracks and crevices and release the medication over time as the skin needs it. A disproportionate accumulation of substances in the dermis and epidermis can be prevented by the microsponge system. Microsponges are specialized polymeric delivery systems comprising porous microspheres that carry a wide range of active ingredients, including sunscreen, emollients, fragrances, essential oils, and anti-infective, anti-fungal, and anti-inflammatory drugs.

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2. Effect of Fermentation on Sensory Attributes of Proso Millet Dhokla (*Panicum Milliaceum*)

Anitha Tendulkar C. M.

PG student,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Manasa R.

Research Scholar,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Shekhara Naik R.

Professor and Head,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Mahesh Shivananjappa

Assistant Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Abstract:

Fermentation technique is known to significantly enhance nutrients in foods. Dhokla is a fermented food of India and is popular throughout the country. Proso millet is an energy packed grain and it is rich in essential nutrients like protein, vitamins, calcium and dietary fibre, being rich in dietary fibre with low glycemic index reduces the risk of type-2 diabetes, it is rich in lecithin which helps in functioning of nervous system, it lowers bad cholesterol. Lactic acid bacteria is the principal microorganism present in Dhokla. This study was conducted to develop and evaluate fermented Dhokla of six different compositions P1, P2, P3, P4, P5 and P6 containing Bengal gram dhal, salt, curd with different proportions of Proso millet (0%, 20%, 40%, 60%, 80%, 100%) were developed and these developed

products were analysed for sensory attributes (n=30). Further, standardization of fermentation time and analysis of proximate composition was also carried out. Fermentation of the developed product was optimised and detected by performing variations in fermentation time (6 - 24 h), followed by their sensory evaluation. Dhokla prepared with 60% Proso millet fermented for 18 h had the highest scores in terms of sensory scores. Dhokla prepared from Proso 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:

Fermentation, lactic acid bacteria, glycemic index, dietary fibre, lecithin, Volume

2.1 Introduction:

Dhoklais a Pulse based fermented product made from fermented rice and Bengal gram dhaleaten for breakfast, main course, side dish or snack and is usually tangy and slightly sweet in taste and different types of Dhoklas are Khatta Dhokla, Rasia Dhokla, Khaman Dhokla, Rava Dhokla. Fermentation increases folic acid, raise the batter volume and gives sponginess to the product. Fermentation reduces tannins, phytic acid and increases total biogenic amines Fermented foods plays an important role in the diet. Fermentation Improves nutrient content, retains enzymes, vitamins and other nutrients, makes food easier to digest and the nutrients easier to assimilate, Enhances Organoleptic properties of food, reduces cooking time, helps in maintaining the healthy configuration of GI micro biota (1). Millets are gluten freehave high energy value, protein and macro nutrient content compared to cereals, Rich in dietary fibre, Niacin in millet lowers the cholesterol, helps to prevent type 2 diabetes, effective in reducing blood pressure, reduces risk of gastrointestinal condition like gastric ulcers and colon cancer (2). Proso millet (Panicum miliaceum L.), also called as white millet, Warm seasonal grass belongs to family poaceae , Rich in nutrients like protein, vitamins and dietary fibre, Gluten free and has low glycemic index , Reduces the risk of type-2 diabetes, Rich in lecithin which supports neural health system (3).

Figure 2.1: Prosomillet (*Panicum Milliaceum*)

2.2 Objective

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

2.3 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 were procured from local market of Mysuru as Proso millet, bengal gram dhal, curd, mustard seeds and salt.
- **Method of preparation:** The cleaned sorghum 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 12hrs 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 30min, then they were cut into square pieces. They were seasoned with oil, mustard seeds, asafoetida, curry leaves and green chillies, for this mixture sugar was added and followed by adding water to dissolve the sugar. Pour this mixture on the Dhokla (7).
- **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.
- **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 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.
- **Sensory Analysis of Prepared Dhokla:** 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 average score of the 20 semi trained panellists by using 9-point hedonic scale.
- **Nutritional Analysis of Prepared Dhokla:** 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 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 a good precision and accuracy (4).

- **Statistical Analysis:** Each sample was analysed in triplicates. The data obtained was analysed statistically using standard methods given by Snedecor and Cochran (5) and by Duncan's multiple range test with the p (0.05 consider to be significant (6).

2.4 Formulation of The Product:

Table 2.1: Formulation of The Product (Ingredients G/100g) For Preparation of Proso Millet Dhokla

Ingredients	P1 (control)	P2 (20%)	P3 (40%)	P4 (60%)	P5 (80%)	P6 (100%)
Proso millet (g)	-	20	40	60	80	100
Bengal gram dhal (g)	100	80	60	40	80	-
Curd (ml)	20	20	20	20	20	20
Salt (g)	2	2	2	2	2	2
Mustard seeds (g)	2	1	1	1	1	1
Curry leaves (g)	5	5	5	5	5	5
Sugar (g)	15	15	15	15	15	15
Chilli (g)	2	2	2	2	2	2

Cleaned Proso 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 2.2: Flow chart for preparation of Proso millet Dhokla

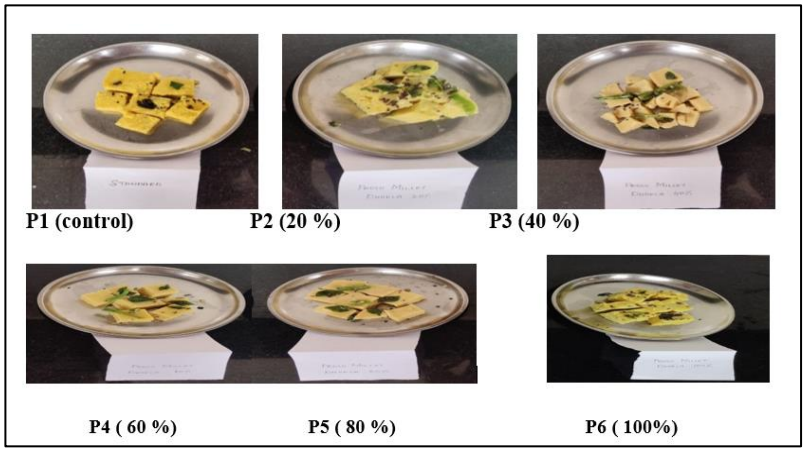

Figure 2.3: Different variations of Dhokla developed from Proso millet in comparision with bengal gram dhal

2.5 Result and Discussion:

2.5.1 Sensory Evaluation of Proso Millet Dhokla:

The study was undertaken to prepare millet based Dhokla by partially replacing wheatflour with Prosomillet flour. The data pertaining to the effect of incorporation of various levels of PMD (20, 40, 60, 80 and 100%) on sensory attributes of Dhoklaand the results are shown in Table 2.2. 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 P4 variation was incubated for different fermentation time to study the optimum fermentation time of Proso millet incorporated Dhokla.

Table 2.2: Sensory evaluation of different variation of Dhokla developed from Proso millet flour with partial replacement of Bengal gram dhal.

Variation	Appearance	Colour	Texture	Taste	Flavour	Overall acceptability
P1 (control)	8.2 ± 0.41	8.3 ± 0.14	8.52 ± 0.16	8.4 ± 0.12	8.2± 0.16	8.2 ± 0.15
P2 (20%)	8.2 ± 0.32	7.9 ± 0.23	7.7 ± 0.12	7.8 ± 0.26	7.8± 0.23	7.9 ± 0.12
P3 (40%)	7.4 ± 0.32*	7.2± 0.23*	6.8 ± 0.12*	7.1± 0.36*	7.1±0.26*	7.4 ± 0.6*
P4 (60%)	7.9 ± 0.24	7.8 ± 0.33	7.9 ± 0.41	7.9 ± 0.41	7.7± 0.26	7.8 ± 0.63
P5 (80%)	7.4 ± 0.14*	7.5 ± 0.14*	7.1 ± 0.36*	7.2± 0.14*	7.0±0.13*	7.2± 0.36*
P6 (100%)	7.3 ± 0.22*	7.5 ± 0.31*	7.1 ± 0.42*	7.2± 0.36*	7.2±0.12*	7.3± 0.13*

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

2.5.2 pH:

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.

Table 2.3: pH of Dhokla Batters

Variation	**Initial pH**	**pH after 6 h**	**pH after 12 h**	**pH after 18 h**	**pH after 24 h**
P1 (control)	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

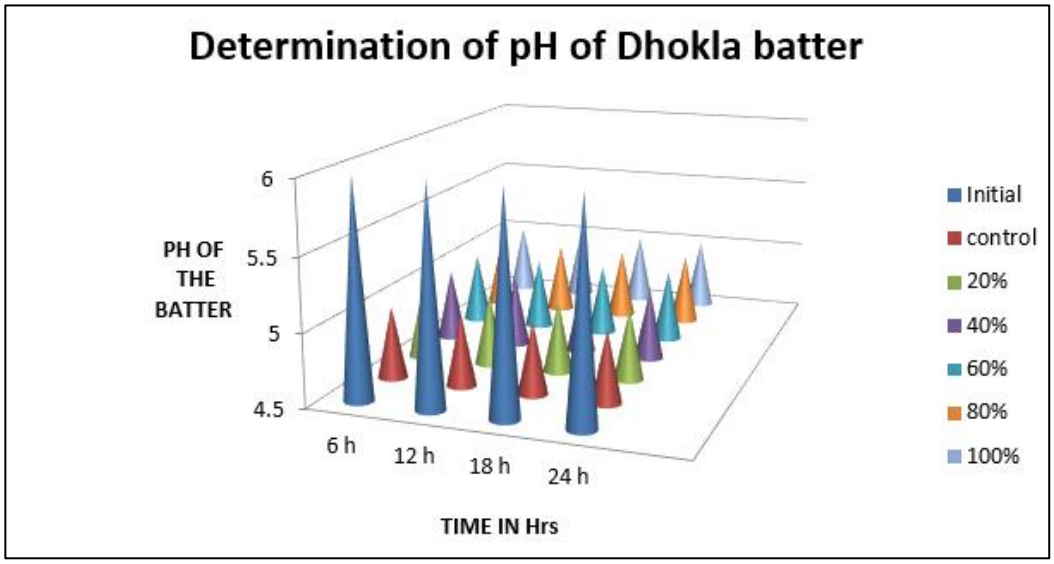

Figure 2.4: pH of Dhokla Batter

2.5.3 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 2.4: Determination of Volume of Dhokla batter

Variations	Initial volume (ml)	volume after 6 h (ml)	Volume after 12 h (ml)	Volume after 18 h (ml)	Volume after 24 h (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

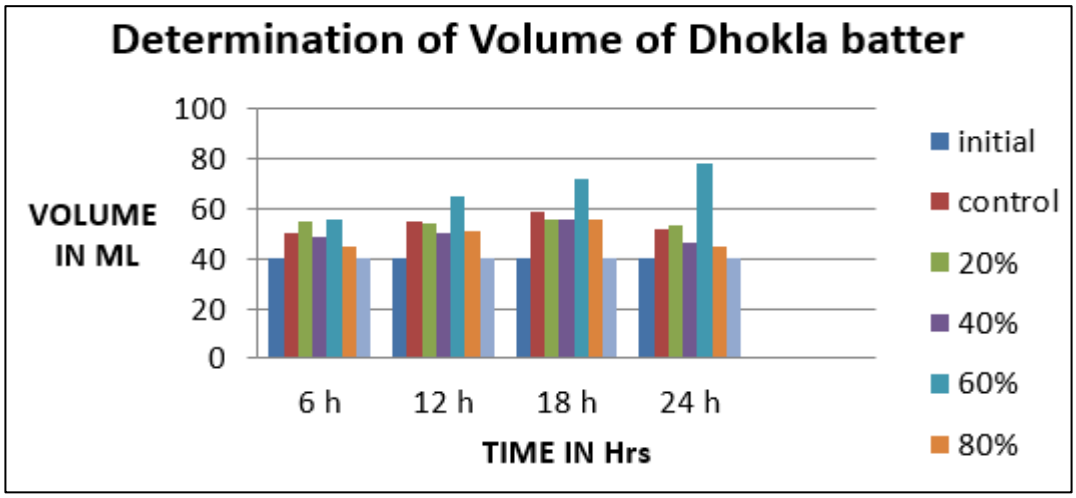

Figure 2.5: Volume of Dhokla Batters

2.5.4 Optimization of Batter Fermentation:

The data pertaining to the effect of incubation ofdifferent fermentation time of Proso millet grains (6hrs, 12hrs, 18hrs, 24hrs). The sensory attributes of Dhokla and the results are shown in Table 2.5. The scores obtained for all sensory attributes of PM1, PM2, PM3 and PM4. The variation PM3 was more acceptable in terms of sensory attributes.

Table 2.5: Sensory evaluation of different variation of Dhokla developed from Proso millet flour with partial replacement of Bengal gram dhal by varying fermentation time.

Attributes	PM1 (6h)	PM2 (12h)	PM3 (18h)	PM4 (24h)
Appearance	8.0 ± 0.12	8.66 ± 0.6	8.9± 0.35	8.30 ± 0.5*
Colour	7.61 ± 0.16*	8.86 ± 0.4	8.46 ± 0.23	7.20 ± 0.5*
Texture	7.72 ± 0.13*	8.99 ± 0.23	8.93 ± 0.9	7.33 ± 0.6*
Taste	7.61 ± 0.22*	8.54 ± 0.36	8.73 ± 0.9	7.43 ± 0.5*
Flavour	7.29 ± 0.32*	8.06 ± 0.76	8.36 ± 0.8	7.33 ± 0.5*
Overall acceptability	8.32 ± 014	7.13 ± 0.32*	8.43 ± 0.9	7.23 ± 0.5*

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

Figure 2.4: Proso Millet Incorporated Dhokla Developed by Varying Fermentation Time

2.5.5 Nutritional Analysis of Prepared Dhokla P4 (60%):

The proximate composition of acceptable Proso millet Dhokla (P4) and that of control were analysed and the results of the same are shown in Table 2.6. The moisture content of all the variations of Dhokla was similar. The values of protein, dietary fibre and calcium content was higher in P4 than that of control, whereas carbohydrate was less. However, ash, iron and phosphorous content were increased in Proso millet Dhokla.

Table 2.6: Nutritional composition of selected variation (P4) of Dhokla developed from Proso millet flour with partial replacement of wheat flour.

Nutrients/100g	Control (BGD)	P4 (60%)
Moisture (%)	20.56 ± 0.36	21.6± 0.12
Carbohydrates (g)	56.00 ± 0.15	53.0 ± 0.11
Protein (%)	14.17 ± 0.12	18.1 ± 0.21*
Fat (%)	4.82 ± 0.32	3.5 ± 0.16
Crude fibre (%)	0.56 ± 0.10	0.8 ± 0.23*
Ash (%)	3.4 ± 0.02	2.0± 0.12
Energy (kcal)	373 ± 0.42	361 ± 0.33*
Iron (mg)	4.43 ± 0.13	5.0 ± 0.13*
Phosphorous (mg)	196 ± 0.28	265 ± 0.22*

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

2.6 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 P4 (18 h). Proso millet Dhokla of 60% (P4) 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, P3 (6 h) had lowest sensory scores and the last variation was also not good due to hyper-fermentation.

Proso 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 Proso millet incorporated Dhokla was found to be 18h and acceptable upto 60%.

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3. Anti-Microbial Activity of Malabar Nut *(Adhatoda vasica)*

Apoorva D. S., Deepika M.

PG student,
Department of Food Science and Nutrition
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Pratapa M. G.

Department of Aquatic Animal Health Management,
ICAR- Central Institute of Fisheries Education,
Mumbai, Maharashtra, India

Manasa R.

Research Scholar,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Shekhara Naik R.

Professor and Head,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Mahesh Shivananjappa

Assistant Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Abstract:

Adhatoda vasica, commonly known as Malabar nut (MN) or Vasaka, belongs to the family Acanthace. MN is a traditionally well-known plant in Ayurveda and unani medicine. The plant parts used for treatment of various diseases like cough, bronchitis, asthma. This plant

can exhibit Anti-asthmatic, Anti-diabetic, Anti-ulcer, Anti-tubercular, Anti-cancer and antimicrobial activity. This review explains the antimicrobial activity of MN. Antimicrobial activity is the process of inhibiting the growth and activity of microorganism such as bacteria and fungi. The antimicrobial activity of MN leaves was studied by agar well diffusion method against P. aeruginosa, K. pneumoniae, S. typhi and S. aureus with Methanolic, Chloroform and Hexane extract on keeping ciprofloxacin as constant and commercially available antibacterial agent. The zone of inhibition was found to be maximum in methanolic extract (17.4 mm for 100 mg/ml) than that of chloroform and hexane. The Ethanolic extract showed maximum zone of inhibition (13.1mm for 1000 µg/ml) when compared to acetone. Ethyl acetate effectiveness was more while petroleum ether showed minimum inhibition on K. pneumoniae, E. coli, S. aureus, E. faecalis. Therefor MN leaves can be a Potential herbal alternative to synthetic anti-microbial agent.

Keywords:

Ciprofloxacin, Ethyl acetate, Anti- asthmatic, S.aureus.

3.1 Introduction:

Malabar nut is the medicinal plant belongs to the species Adhatoda vasica. In Sanskrit it is called as Vasaka, Adusoge in kannada & Ya-Zui-Hua in Chinese [1]. The plant is distributed throughout India especially in lower Himalayas and has been used in the indigenous system of medicine for 2000 years [2]. It can grow up to the altitude 1300 meter above sea level [3]. In Ayurveda, a preparation made from Vasaka flowers, known as gulkand is used to treat tuberculosis. MN plant as beneficial effects like in the treatment of infectious diseases while simultaneously alleviate, many of the side effects of synthetic anti-microbials [4].

The leaves are used to treat malarial fever, chronic fever, intrinsic haemorrhage, cough, asthma, leprosy, skin diseases, and piles [5]. The essential oil of this plant is chiefly responsible for the expectorant action [6] [7].

The volatile oils of MN were used for their antibacterial properties against 25 genera of bacteria by using agar well diffusion technique [8]. It is a rich in source of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils [9]. The current investigation aimed to explore the Malabar nut antimicrobial activity of different extract using different bacteria.

3.2 Morphology:

The leaves of MN are arranged opposite praise lanceolate; edge whole & grow 1.226 m leaves in length. The colour of leaf is light green on top and dark green on beneath [10].

Flower are zygomorphic, hypogynous, white colour and calyx of MN flower have 5 lobed with large white petals, streaks are purple are purple colour on lower lip (11). The fruits are longitudinally channelled capsule to 2.5 cm long and 0.8cm wide and seeds globular and rugose (12).

3.3 Phytochemical Composition:

MN is a rich source of several alkaloids, essential oil, sugar, amino acid, fat, protein and vitamin. The leaves are composed of major constituents Viz., Vasicoline, Adhatodine, Vasicolinine, Anisotine, Vasicine, and Vasicinone. It is also containing vitamin C in large amount with ß- Carotene and essential oil. Roots contain vasicinolone, vasicol, pegamine & sitosterol. beta glucoside galactose and 2-hydroxyl-4-glucoyl-oychalcone are also found in the roots (13). The flowers are rich source of 2,4 dihydroxy chalcone, glucoide, Quercetin and kaempferol, triterpenes (a-amirine) (14).

3.4 Nutritional Composition:

100 g of MN plant has 68.25 g of carbohydrate. It is a poor source of ash 2.18 % and Chromium 4 mg. It is a rich source of calcium 6808 mg, potassium 3110 mg, magnesium 482 mg, Iron 70 mg, protein 6.12 %, Fat 19 % and 6.5 % of crude fiber (15).

3.5 Pharmacological Activities:

The Malabar nut plant contains various bioactive compounds that are responsible for its pharmacological activity. Some of the pharmacological activities of Malabar nut are anti-asthmatic, antidiabetic, antitubercular, antiulcer, hepatoprotective and wound healing (8).

3.5.1 Antimicrobial Activity of Malabar Nut:

Yarapa Lakshmikantha Ramachandra et al., in 2013 studied the antibacterial activity on *p. aeruginosa, K. pneumoniae, S. aureus* and *S. typhi* by agar well diffusion method using different extracts such as methanol, chloroform and hexane. The methanolic extract showed high zone of inhibition 17.1 mm ,17.0 mm, 18.1 mm, 17.5 mm for 100 mg/ml was compared to chloroform and hexane, exhibited moderate to weak activity respectively. On comparison with standard ciprofloxacin's potential against Bacterial growth, showed 23.83 mm, 23.17 mm, 24.8 mm and 21.90 mm for 50 mg/ml of zone of inhibition respectively [16]. A.K Sarker et al., in 2011 determined the Antimicrobial activity of MN leaves by disc diffusion technique using nutrient agar medium. The microorganism's viz., *E. coli, S. typhi, B. subtilis* and *S. aureus* was extract with essential oil and ethanol in ratio 5:1. The zone of inhibition was 16.0 mm, 7.5 mm, 2 mm and 6.0 mm for 50 µg/ disc. The Antimicrobial effect of essential oil was found to be declined with dilution [17].

The antimicrobial activity of MN leaves was screened by agar well diffusion assay using Muller Hinton agar plates. Then ethanolic extract against *S. aureus, S. epidermidis, B. subtilis, P. vulgaris* and *C. albicans* showed maximum zone of inhibition. Similarly, the petroleum ether extract showed 16 mm of inhibition zone on S. aureus, other tested organism against petroleum ether and aqueous extract was not effective.

The MIC of ethanolic extract was ranged between 12-13 mm for 100 mg/ ml against *S. aureus, S. epidermidis, B. subtilis, P. vulgaris* and *C. Albicans*. The MIC of petroleum ether was found to be 13 mm for 100 mg/ ml on *S. Aureus. This study was conducted by* A. Karthikeyan et al., 2008 [18].

Mohd Yusuf et al., in 2016 studied the antimicrobial activity of Malabar nut leaves. The antibacterial assessment was carried out by disc diffusion assay method against *K. pneumoniae, E. coli, S. aureus* and *E. faecalis* using different solvents are ethanol, acetone, ethyl acetate and petroleum ether, Then as a result ethanol extract was showed 12.5 mm, 10.5 mm, 13.9 mm and 15.5 mm for 1000 µg/ ml respectively. This study was proved MN plant possess synergized potentiality for biological characteristics [19].

The isolated ethyl acetate compound in the flower showed antibacterial activity against *S. typhi. E. coli, E. faecalis* and *B. cereus* showed inhibition zone was 17 mm, 15 mm, 16 mm and 16 mm for 40 mg/ ml respectively, when tested organism was compared with standard chloramphenicol showed 19 mm, 21 mm, 22 mm and 21 mm of inhibition zone for 1 mg/ ml. The antifungal activity against *C. lunata* and *C. albicans* using ethyl acetate extract showed zone of inhibition 16 mm and 15 mm for 40 mg/ ml, when compared with standard drug fluconazole, showed 21 mm and 19 mm for 1 mg/ml of zone of inhibition. This experiment was conducted by N. Muruganantham et al., in 2015 [20].

Table: 3.1 Antimicrobial Activity of Malabar Nut

Sr. No	Model	Extract	Result (zone of inhibition in mm)	Reference
1	*P. aeruginosa*	ME (100 mg/ml) CE (100 mg/ml) HE (100 mg/ml) Ciprofloxacin (50 mg/ml)	17.1 12.8 11.1 23.8	[16]
		EE (mg/ml) PEE (mg/ml) Water (mg/ml) Positive control (mg/ml)	15 - - 10	[18]
2	*K. pneumoniae*	ME (100 mg/ml) CE (100 mg/ml) HE (100 mg/ml) Ciprofloxacin (50 mg/ml)	17.0 12.3 10.3 23.1	[16]
		EE (1000 µg/ml) AE (1000 µg/ml) EAE (1000 µg/ml) PEE (1000 µg/ml) Ceftriaxone (1000 µg/ml)	12.5 9.5 9.7 9.8 17.5	[19]
		EE (mg/ml) PEE (mg/ml) Water (mg/ml) Positive control (mg/ml)	- - - 16	[18]
3	*S. typhi*	ME (100 mg/ml) CE (100 mg/ml)	17.5 13.1	

Sr. No	Model	Extract	Result (zone of inhibition in mm)	Reference
		HE (100 mg/ml) Ciprofloxacin (50 mg/ml)	11.5 21.9	[16]
		EAE (40 mg/ml) Chloramphenicol (1 mg/ml)	17 19	[13]
		Essential oil: EE 5:1 (50 µg/disc) Ampicillin (10 µg/disc)	7.5 25.0	[17]
4	*S. aureus*	ME (100 mg/ml) CE (100 mg/ml) HE (100 mg/ml) Ciprofloxacin (50 mg/ml)	18.1 14.1 11.8 24.8	[16]
		EE (1000 µg/ml) AE (1000 µg/ml) EAE (1000 µg/ml) PET (1000 µg/ml) Ceftriaxone (1000 µg/ml)	13.9 10.2 10.1 10.5 19.1	[19]
		EE (mg/ml) PEE (mg/ml) Water (mg/ml) Positive control (mg/ml)	19 16 - 20	[18]
		MIC EE (100 mg/ml) PEE (100 mg/ml)	 12 13	
		Essential oil: EE 5:1 (50 µg/disc) Ampicillin (10 µg/disc)	6.0 24.0	[17]
5	*E. coli*	EE (1000 µg/ml) AE (1000 µg/ml) EAE (1000 µg/ml) PEE (1000 µg/ml) Ceftriaxone (1000 µg/ml)	10.5 7.6 8.2 8.8 16.9	[19]
		EAE (40 mg/ml) Chloramphenicol (1 mg/ml)	15 21	[13]
		EE (mg/ml) PEE (mg/ml) Water (mg/ml) Positive control (mg/ml)	- - - 13	[18]

Sr. No	Model	Extract	Result (zone of inhibition in mm)	Reference
		Essential oil: EE 5:1 (50 µg/disc) Ampicillin (10 µg/disc)	16.0 30.0	[17]
6	*E. faecalis*	EE (1000 µg/ml) AE (1000 µg/ml) EAE (1000 µg/ml) PEE (1000 µg/ml) Ceftriaxone (1000 µg/ml)	15.5 11.1 12.5 11.6 28.5	[19]
		EAE (40 mg/ml) Chloramphenicol (1 mg/ml)	16 22	[13]
		EE (mg/ml) PEE (mg/ml) Water (mg/ml) Positive control (mg/ml)	- - - 16	[18]
7	*B. cereus*	EAE (40 mg/ml) Chloramphenicol (1 mg/ml)	16 21	[13]
8	*C. lunata*	EAE (40 mg/ml) Chloramphenicol (1 mg/ml)	16 20	[13]
9	*C. albicans*	EAE (40 mg/ml) Chloramphenicol (1 mg/ml)	15 19	[13]
		EE (mg/ml) PEE (mg/ml) Water (mg/ml) Positive control (mg/ml)	12 - - 14	[18]
		MIC EE (100 mg/ml)	 12	
10	*S. epidermidis*	EE (mg/ml) PEE (mg/ml) Water (mg/ml) Positive control (mg/ml)	18 - - 19	[18]
		MIC EE (100 mg/ml)	 12.5	
11	*B. subtilis*	EE (mg/ml) PEE (mg/ml) Water (mg/ml) Positive control (mg/ml)	14 - - 16	[18]

Sr. No	Model	Extract	Result (zone of inhibition in mm)	Reference
		MIC EE (100 mg/ml)	 13	
		Essential oil: EE 5:1 (50 µg/disc) Ampicillin (10 µg/disc)	 2 16.0	[17]
12	*P. vulgaris*	EE (mg/ml) PEE (mg/ml) Water (mg/ml) Positive control (mg/ml)	15 - - 16	[18]
		MIC EE (100 mg/ml)	 12	

P. aeruginosa = Pseudomonas aeruginosa. K. pneumoniae= Klebsiella pneumoniae, S. typhi= Salmonella typhi. S. aureus= Staphylococcus aureus. E. coli= Escherichia coli. E. faecalis = Enterococcus faecalis. B. cereus= Bacillus cereus, C. lunata = Cochliobolus lunataus. C. albicans = Candida albicans. B. subtilis= Bacillusmsubtilis, S. epidermidis = Staphylococcus epidermidis. P. vulgaris = Pemphigusvulgaris, EE= Ethyl extract, AE= Acetone extract, EAE= Ethyl acetate extract, PEE= Petroleum ether extract, ME= Methyl extract, CE= Chloroform extract, HE = Hexane extract, EE= Ethanol extract, EAE= Ethyl acetate extract, PET= Petroleum ether extract. (-) = No activity, MIC= Minimum inhibitory concentration.

3.6 Conclusion:

The MN leaves and flower showed potential Antimicrobial activity on variety of microorganism with different extracts Viz., ethanol, methanol, hexane, petroleum ether, & ethyl acetate extract etc. The essential oil shows potential antimicrobial activity against *E. coli* exhibits highest zone of inhibition 16.0 mm for (50 µg/ml) responsible for food poisoning. The ethanolic extract showed maximum zone of inhibition 13.1 mm for (1000 µg/ml) when compare to acetone ethyl acetate effectiveness was more while petroleum ether showed minimum inhibition on *K. pneumoniae, E. coli, S. aureus, E. faecalis.* Thus, MN can be used as an eco-safe and effective treatment for bacterial infections.

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4. Effect of Fermentation Time on Sensorial Attributes of Brown Top Millet (*Urochloa Ramosa*) Idli

Bhoomika B. M.

PG student,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Manasa R.

Research Scholar,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Shekhara Naik R.

Professor and Head,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Mahesh Shivananjappa

Assistant Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Abstract:

Idli is a cereal-legume-based fermented food, which is widely consumed in India. Idli is prepared using Black gram dhal and parboiled rice. The fermentation of idli demonstrates a leavening action caused by the Lactic acid bacterium. Micro-organism Leuconostoc mesenteroides is responsible for souring and gas production in the batter. Idli makes an important contribution to the diet as a source of protein, calories, and vitamins, especially B-complex vitamins. Brown top millet is a minor millet, minor millets are a highly valuable natural resource.

They contain all essential nutrients and can be used as a functional food. Brown top millet is a great source of protein, dietary fiber, iron, and zinc. It also acts as an excellent medicine in dealing with lifestyle diseases. In this study, idles were prepared in six variations B1, B2, B3, B4, B5, and B6 containing parboiled rice, Black gram dhal, with the incorporation of brown top millet in different portions (0%, 20%, 40%, 60%, 80%, 100%). These variations were analyzed for sensorial properties (n-15). Sensory evaluation was done by semi-trained panelists using the hedonic scale method. Among all these variations of idli (B4) had more acceptability. The present study documents how microbial growth influences the batter characteristics and quality of idli in terms of sensory attributes.

Keywords:

Leavening action, sensorial properties, hedonic scales

4.1 Introduction:

Idli is a popular food product prepared from a fermented batter of rice (Oryza sativa L.) and black gram dhal, idli originated in south India and had gained popularity throughout India and it's a part of global cuisine due to its soft spongy texture, characteristic sour taste, and an attractive aroma. Apart from its organoleptic properties, it is highly nutritive and a significant source of calories, proteins, and micronutrients. Fermentation of the batter causes an increase in the protein efficiency ratio, essential amino acids, and vitamins such as niacin, riboflavin, and thiamine, together with a decrease in anti-nutrient content.

The total time required for the preparation of idli is about 15–18 h including washing, soaking, grinding, fermenting, and steaming (1). Fermentation leads to nutritional enhancement of the idli batter. It leads to the formation of bioactive compounds such as vitamins (B12, B1, B2, and folic acid) methionine, choline, etc. (2,3,4).

As rice-based diets are deficient in B-group vitamins, Vitamin B increase due to fermentative changes that occurs during batter fermentation (5).

Fermentation also reduces the anti-nutrients such as phytate, protease (trypsin and chymotrypsin) inhibitors, haemagglutinin, etc. (3,4,6).

As the digestibility of protein and starch is limited due to the presence of anti-nutrients, reduction in anti-nutrients is important in idli batter fermentation. Millets are the traditional crops cultivated by smallholders and tribal farmers mainly under rain fed conditions.

These are among the foremost ancient cultivated crops In India. Millets are suitable crops for dry land rain-fed regions (7). Millets are considered as crop of food security because of their sustainability in adverse agro-climatic conditions (8).

Along with nutrition millets offer health benefits in daily diet and help in the management of disorders like diabetes mellitus, obesity, hyperlipidemia, etc (9). Browntop millet (Urochloa ramose L.) is also known as 'korale' in Karnataka. It is the underutilized millet, which is neglected by the mono-crop-based agriculture system.

It originated from Southeast Asia and is presently grown in Africa, Western Asia, Arabia, China, and Australia (7). Brown top millet can grow with either a compact or open panicle and can have either shattering or Indehiscent spikelet (10). Brown top millets are used for domestic consumption or product development with optimum nutrients. There are very few studies conducted on the effect of processing on the physicochemical properties of browntop millet. Hence, there is a need to explore the potentiality and utility of grain in daily diet by demonstrating the suitability of the best processing methods with optimal physicochemical properties for consumption (11).

4.2 Objectives:

- To formulate idli using brown top millet
- To study the effect of fermentation time on the sensorial attributes of the product
- To develop protein and fiber-rich food product

4.3 Materials and Methods:

A. 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, browntop millet, Black gram dhal, and salt were procured from a local grocery shop in Mysuru.

B. Methods:

- **Preparation of Browntop Millet (BT) Idli:**
  Idli was prepared by replacing parboiled rice with BT millet in different proportions. Parboiled rice, BT, and black gram dhal were soaked for 4 hours. Parboiled rice and BT were ground coarsely and black gram dhal was ground into a fine paste. Combine both the slurries into a thick batter and mix well by adding salt. Allow the batter to ferment for 12 hours in a warm place (34-37 °C). Pour the fermented batter into small cups of idli cooker, and steam it for 20 minutes.
- **Optimization of Batter Fermentation:**
  After the addition of 2% salt and total weighed raw material, the batter of selected variation was allowed to ferment for different time periods (6, 12, 18, 24 h) in a stainless-steel vessel. The temperature during the process of fermentation was not controlled
- **pH and Volume:**
  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.
- **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. Nutritional analysis of idli:

Standard AOAC (1980) method was used to determine the nutritional composition of selected variation (B4) of BT 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).

D. 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).

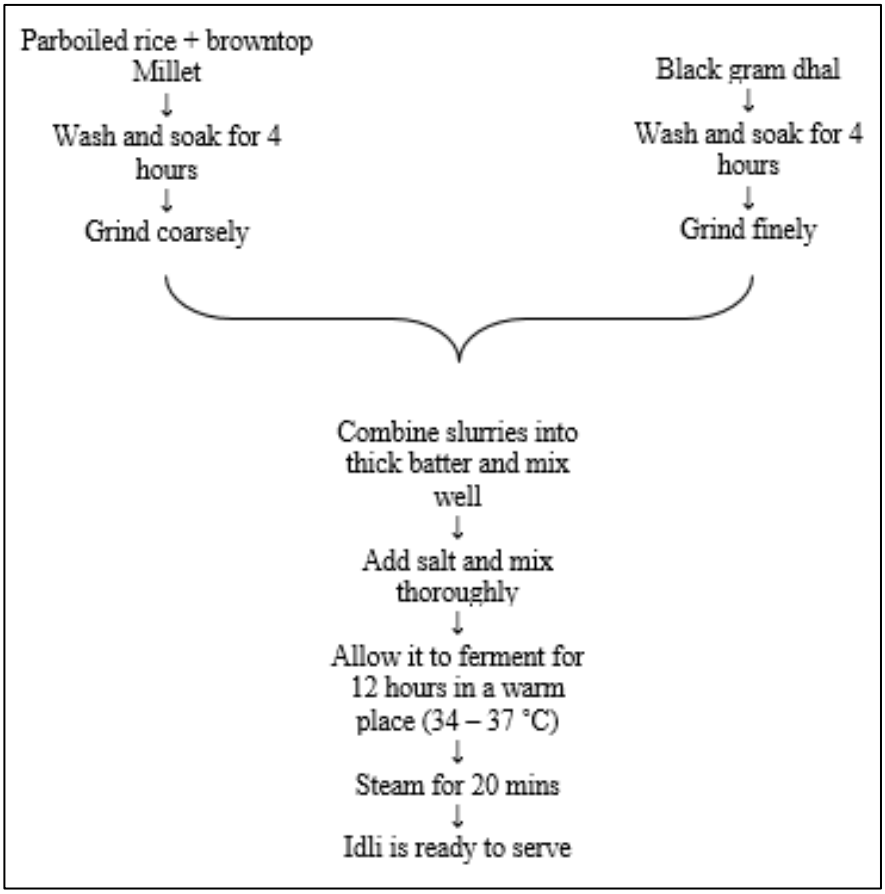

Figure 4.1: Flow Chart for Preparation of BT idli

4.4 Formulation Of the Product:

Table 4.1: Formulation of Ingredients (g/100 g) for Preparation of BT idli

Ingredients	B1 (Standard)	B2 (20%)	B3 (40%)	B4 (60%)	B5 (80%)	B6 (100%)
Brown top 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

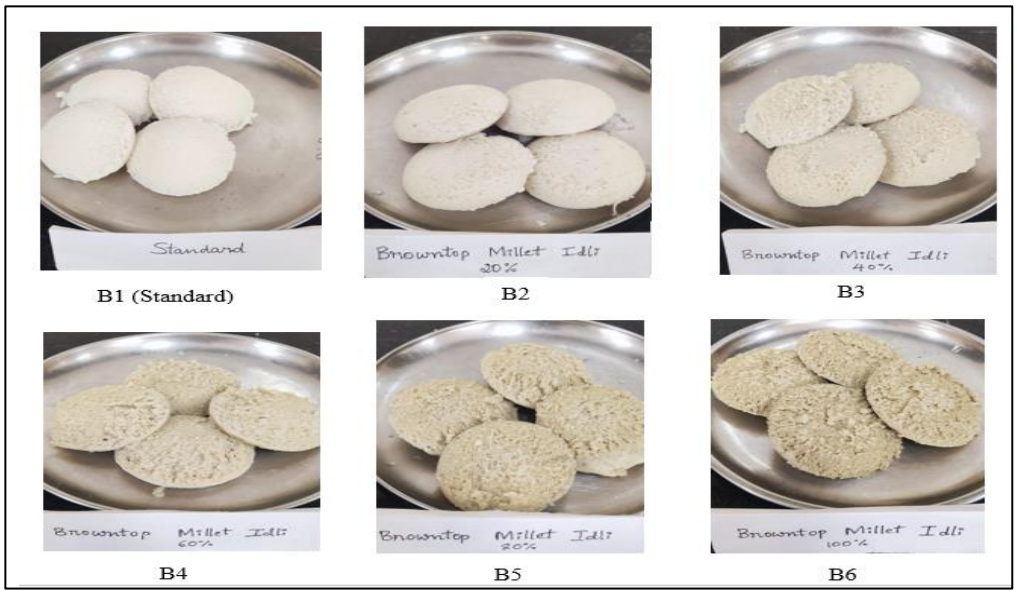

Figure 4.2: Different Variations of Idlis Developed from BT Millet In Comparison to Parboiled Rice Idli

Figure 4.3: BT Millet Incorporated Idli Prepared By

4.5 Results and Discussion:

This study helps to evaluate the idli prepared by different variations and fermentation time brown top millet idli which possess a low glycaemic index.

The data pertaining to the effect of the incorporation of various levels of brown top millet (20 %, 40 %, 60 %, 80 %, and 100 %) on sensory attributes of idli and the results are shown in Table 2. Idli prepared by incorporating 60 % brown top millet had the highest scores in terms of sensory evaluation.

4.5.1 Sensory Evaluation of BT Incorporated Idli:

This study was undertaken to prepare Idli by partially replacing rice with brown top millet. The data pertaining to the effect of the incorporation of various levels of brown top millet (20 %, 40 %, 60 %, 80 %, and 100 %) on sensory attributes of Idli and the results are shown in Table 4.2. The results showed that B4 (60 %) variation had more acceptability.

The scores obtained for sensory attributes for B2 were almost similar on par with the control. B5 obtained least scores and had less acceptability compared to all other variations.

Table 4.2: Sensory evaluation of different variations of Idli developed from the incorporation of BT with Parboiled rice, Values are mean ± SD, p ≤ 0.05 (Holm Sidak method), n=20

Attributes	B1 (Standard)	B2 (20%)	B3 (40%)	B4 (60%)	B5 (80%)	B6 (100%)
Appearance	8.60±0.50	8.06±0.79	7.0±0*	7.53±0.51*	7.26±0.450*	7.33±0.48*
Color	8.66±0.48	7.73±0.45*	6.43±0.50*	7.6±0.50*	6.8±0.67*	6.0±0*
Texture	8.06±0.45	7.66±0.61	6.73±0.45*	7.86±0.35	7.13±0.63	6.53±0.51*
Taste	8.26±0.70	6.73±0.45	6.73±0.45	7.93±0.25	7.2±0.56	6.6±0.50*
Flavor	8.20±0.67	6.73±0.45*	6.73±0.45	7.93±0.15	7.2±0.56	6.53±0.51*
Overall acceptability	8.26±0.59	6.73±0.45*	6.73±0.45*	8.0±0	7.26±0.45	6.6±0.48*

4.5.2 Optimization of Fermentation Time:

The most accepted variation B4 (60 %) was optimized for the fermentation time by varying the fermentation time (6 to 24 hours).

The data pertaining to the effect of different fermentation times on brown top millet idli and the results are shown in Table 4.3.

The result showed that the optimum fermentation time for brown top millet incorporated idli was found to be 12 hours.

Table 4.3: Sensory scores of Idlis prepared with BT incubated for different fermentation Time

Attributes	Standard	6 h	12 h	18 h	24 h
Appearance	8.60±0.50	6.0±0*	8.13±0.35	7.3±0.41*	6.6±0.50*
Color	8.66±0.48	6.2±0.41*	8.13±0.35*	7.23±0.41*	6.13±0.74*
Texture	8.06±0.45	6.13±0.35*	8.06±0.25	7.73±0.70	6.0±0.53*
Taste	8.26±0.70	6.2±0.41*	8.13±0.35	7.23±0.41	5.33±0.48*
Flavor	8.20±0.67	6.2±0.45*	8.06±0.25	6.21±0.45*	5.73±0.45*
Overall acceptability	8.26±0.59	6.2±0.45*	8.13±0.35	6.21±0.45*	5.66±0.48*

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

4.5.3 Ph Value:

The pH of the BT idli batter was measured using the pH paper. The fermented BT idli batter of the selected variation (12 h) showed a pH of 5, which shows the acidic nature.

Acidic pH in idli is primarily due to the fermentation process. Lactic acid fermentation is mainly responsible for the sour taste and acidic pH of idli batter.

The lactic acid bacteria present in the batter convert the sugars present in the rice and lentils into lactic acid.

The acidic pH in Idli 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 idli.

Table 4.4: pH of BT incorporated (B4) idli batter fermented for different time periods

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

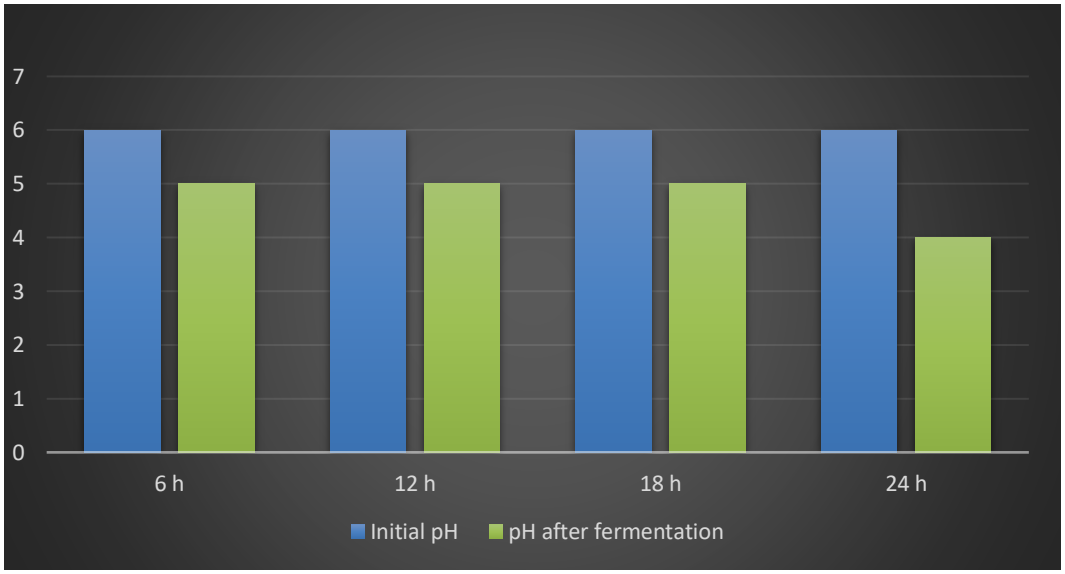

Figure 4.4: Determination of pH of BT Incorporated Idli batter

4.5.4 Volume:

The difference in volume was measured in the BT idli batter before and after the optimum fermentation of the batter. The batter was fermented in a warm place (30 to 32 °C) for 6, 12, 18, and 24 hours.

The volume of the batter of selected variation (12 h) before fermentation was 60 ml and the volume of the batter after fermentation was 81 ml. 21 ml rise in the batter was observed after fermentation.

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 causes the batter to rise, resulting in contributing fluffiness to the idli.

Table 4.5: Volume of BT incorporated (B4) idli batter fermented for different time periods

Variations	Initial volume (ml)	Volume after fermentation (ml)
6 h	60	76
12 h	60	81
18 h	60	87
24 h	60	91

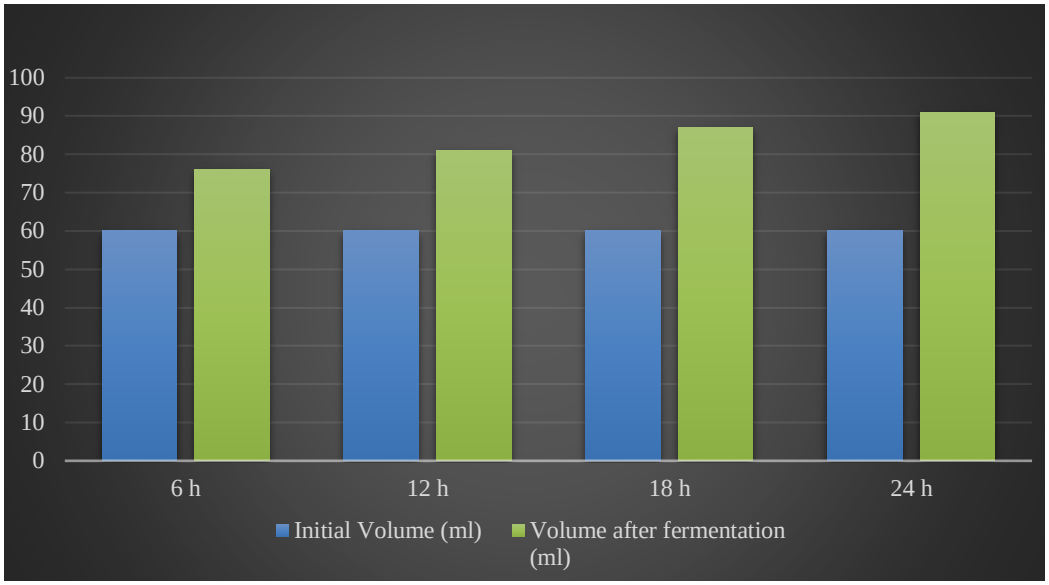

Figure 4.5: Determination of Volume of BT Incorporated Idli batter

4.5.5 Proximate Analysis:

Table 4.6: Proximate analysis of selected variation (B4) of BT incorporated idli

Nutrients/100 g	Standard	BTM
Energy (kcal)	298.81±0.19	319.09±0.18*
Carbohydrates (g)	60.99±0.95	61.54±0.85*
Protein (g)	11.81±1.10	13.72± 0.44*
Fat (g)	0.84±0.35	2.15±0.55*
Ash (g)	1.59±0.02	2.0±0.03*
Crude fibre (g)	1.08±0.03	0.25±0.05
Moisture (%)	23.69±0.15	20.37± 011*
Iron (mg)	1.96±0.12	4.20±0.06*
Phosphorus (mg)	95.37±0.12	165.6±0.66*

Values are mean ± SD, n=3 *p < 0.05 (Holm sidak method)

4.6. Conclusion:

The study was undertaken to prepare idli by partially replacing rice with brown top millet. The data pertaining to the effect of the incorporation of various levels of brown top millet

(20 %, 40 %, 60 %, 80 %, and 100 %) on sensory attributes of idli and the results are shown in Table 2. The results showed that B4 (60 %) variation has the most acceptability. The scores obtained for sensory attributes for B3 were almost similar on par with the control. B5 and B6 showed decreased scores and were less acceptable compared to other variations. The most acceptable variation 60 % was further prepared by varying their fermentation time. The data pertaining to the effect of different fermentation times on brown top millet idli and the results are shown in Table No. 3. The result showed that the optimum fermentation time for brown top millet incorporated idli was found to be 6h and acceptable. The proximate composition of accepted brown top millet idli B4 (60 %) (6 h fermentation time) and that of control were analyzed and the results of the same are shown in Table 6. The values of moisture, protein, and ash content were higher in B4 than that of the control, whereas carbohydrate was decreased. However, Protein, ash, iron, and phosphorus content were rich in brown top millet Idli.

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16. Bhoomika B M, PG student, Dept. of Food Science and Nutrition, Yuvaraja's College (Autonomous), University of Mysore, Mysore Mail ID – bhoomika7900@gmail.com, Mobile no.-8549039388.
17. Manasa R, Research Scholar, Dept. of Food Science and Nutrition, Yuvaraja's College (Autonomous), University of Mysore, Mysore.
18. Shekhara Naik R, Professor and Head, Dept. of Food Science and Nutrition, Yuvaraja's College (Autonomous), University of Mysore, Mysore.
19. Mahesh shivananjappa, Assistant Professor, Dept. of Food Science and Nutrition, Yuvaraja's College (Autonomous), University of Mysore, Mysore.

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5. Antibacterial activity of *Melissa officinalis* (Lemon Balm)

Bhoomika B. M., Anitha Tendulkar C. M.

PG student,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Pratapa M. G.

Department of Aquatic Animal Health Management,
ICAR- Central Institute of Fisheries Education,
Mumbai, Maharashtra, India.

Manasa R.

Research Scholar,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Shekhara Naik R.

Professor and Head,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Mahesh Shivananjappa

Assistant Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Abstract:

Melissa officinalis (MO) is also called as lemon balm; it belongs to the family Lamiaceae. It has been traditionally used for different medicinal purposes including wound healing and

antibacterial activity. The Phytochemical compound citral present in MO plays an important role in antibacterial activity. The Aqueous extract of MO showed maximum microbial inhibition concentrations against E. coli, S. aureus, Vibrio parahaemolyticus and also showed maximum zone of inhibition against S. aureus, E. coli and minimum zone of inhibition against Vibrio parahaemoliticus. The Ethanolic extract of MO showed increased microbial inhibition concentration against E. coli, S. aureus, Bacillus cereus and also showed maximum microbial inhibition concentration against E. coli, S. aureus, Bacillus cereus and then the maximum zone of inhibition was noticed against E. coli, S. aureus along with minimum concentration was observed against Bacillus cereus.

Keywords:

Medicinal herb, E. coli, Phytochemistry, citral, bacteria.

5.1 Introduction:

Melissa officinalis is an herbal medicine indigenous to east of the Mediterranean and central Asia. It is called as Lemon balm. It is used for several therapeutic purposes in the form of tonic, antispasmodic medicine, carminative, diaphoretic medicine, medical dressing for wounds, and sedative. Chemical studies on its composition have shown that it contains mainly flavonoids, terpenoids, phenolic acids, tannins, and essential oil [9]. Indeed, the plant has antioxidant, anti-inflammatory, antispasmodic, antimicrobial, neuroprotective, nephroprotective, antinociceptive effects [10]. MO can be used as flavoring agent in frozen yogurt and refreshment, often in combination with other herbs such as spearmint and it can be used for building up the memory, and also has hypoglycemic, hypolipidemic, antibacterial, antioxidant, antidepressant effects [2-3]. *Melissa officinalis* tea consumption is also beneficial as it increases salivary antioxidants level in smokers [11]. The effect of MO plant is also effective in increasing lipid peroxideative in better states of second degree burn wound healing [12]. The extract of MO also exhibits the anticancer activity in colorectal cancer (CRC) [13]. Than lifestyle modifications alone, MO as a medicinal plant seem to support the higher cardio protective effects [14]. The essential oil of MO exhibits high antimicrobial activity against microorganisms [15].

Figure 5.1: Melissa Officinalis

5.2 Morphological Description:

Lemon balm is a perennial herb and its Stem grows about 0.6 meter (2 feet) tall and grows to a maximum height of 1 m (3 feet 3 in). It is characterized by square stems, with wrinkled

toothed leaves of irregularly heart- shaped. The leaves can be flat or slightly rough leaves. The small flowers (0.5–1.5 cm size) are produced all summer long and they are small ivory or cream-colored or lavender in color and its fruit is dry but does not split when ripened [4].

5.3 Phytochemical Constituents of Lemon Balm:

Table 5.1: Phytochemical Constituents of Lemon Balm [3].

Class of Compounds	Chemical Constituents
Volatile compounds	Geranial, Neral, Citronellal, Geraniol
Triterpenes	Ursolic acid, oleanolic acid
Phenolic compounds	Rosmarinic acid, caffeicacid, protocatechuic acid
Flavonoids	Quercetin, Rhamnocitrin, Luteolin

5.4 Traditional Uses:

Lemon balm leaves have been conventionally used for several medicinal purposes such as antispasmodic, carminative and diaphoretic. It is also used as medical dressing for wounds, and sedative. Lemon balm has a calming effect of the nervous system it is used to relieve anxiety, stress and nervousness. They also help in digestion and relieve from bloating, gas and indigestion. Lemon balm is also used to treats infections caused by bacteria, virus and fungi and they are used to relieve pain and discomfort associated with menstrual cramps. It treats diabetes, depression, obesity and cancer [4].

5.5 Pharmacological Activity of Lemon Balm:

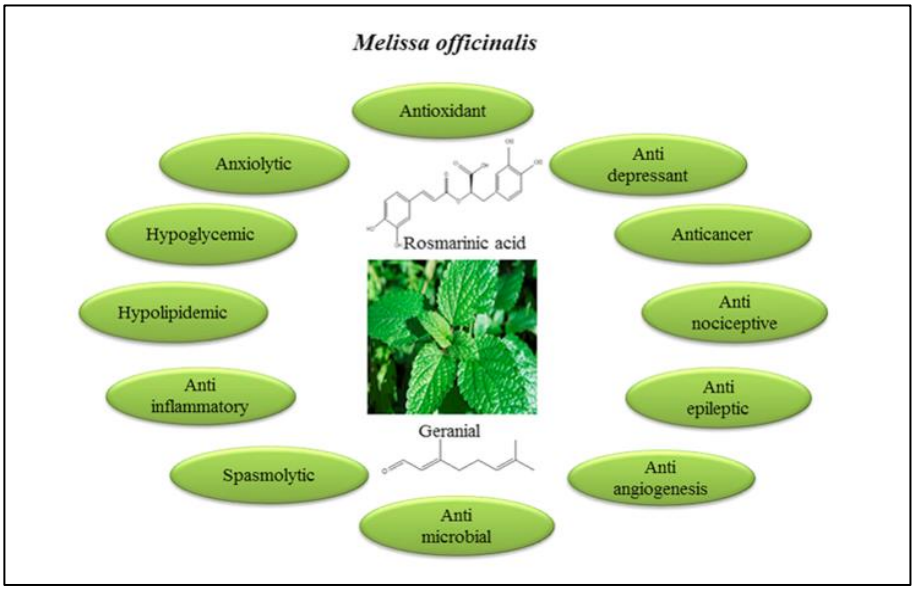

Figure 5.2: Pharmacological Activity of Lemon Balm

5.6 Antibacterial Activity of Lemon Balm in Bacteria:

Fahima and coworkers studied antibacterial activity of lemon balm, as a treatment they used ethanolic extract of 5 ul/ml was given it resulted in 17-18 mm zone of inhibition and 0.13 mm of MIC [5].

Michela and coworkers in 2022 studied antibacterial activity of lemon balm on E. coli, as a treatment Aq Extract 1 ul/ml was used. It resulted in 14-17 mm zone of inhibition and 0.012 mm of MIC [8].

Huijie and coworkers studied the antibacterial activity of lemon balm on Vibrio parahaemolyticus, as a treatment Aq. Extract (1 ul/ml) was used and 1-2 mm zone of inhibition and 0.002 mm MIC was observed in culture of the bacteria [7].

Michela and coworkers studied the antibacterial activity on S. aureus as a treatment Aq Extract (1 ul/ml) was used it resulted in 14-21 mm zone of inhibition and 0.010 mm of MIC [6]. Fahima and coworkers studied the antibacterial activity on S. aureus as a treatment ethanolic Extract (5 ul/ml) was used, as a result 12-14 mm zone of inhibition and 0.12 mm of MIC was observed [5].

Irino and coworkers studied the antibacterial activity on S. aureus was treated with ethanolic Extract (1 ul/ml), as a result 0 mm zone of inhibition and 0.2 mm of MIC was observed [8].

Table 5.2: Antibacterial Activity of Leaves of Lemon Balm in Bacteria

Sr. No	Model	Treatment	RESULT (Zone of inhibition)	MIC (inmm)	References
01	*E. coli*	EE (5 ul/ml)	17-18	0.13	**[5]**
		EE (1 ul/ml)	1-3	0.3	**[8]**
02	*V. parahaemolyticus*	Aq E (1 ul/ml)	1-2	0.002	**[7]**
03	*S. aureus*	AqE (1 ul/ml)	14-21	0.010	**[6]**
		EE (5 ul/ml)	12-14	0.12	**[5]**
		EE (1 ul/ml)	0	0.2	**[8]**
04	*B. cereus*	EE (1 ul/ml)	10	0.03	**[8]**

(E. coli - *Escherichia coli*, EE- Ethanolic extract, AqE – Aqueous extract, MIC – minimum inhibitory concentration. *V. parahaemolyticus* – *Vibrio parahaemolyticus*, S. aureus – *Staphylococcus aureus* , AqE- aqueous extract, B. cereus – bacillus cereus,EE– Ethanolicextract)

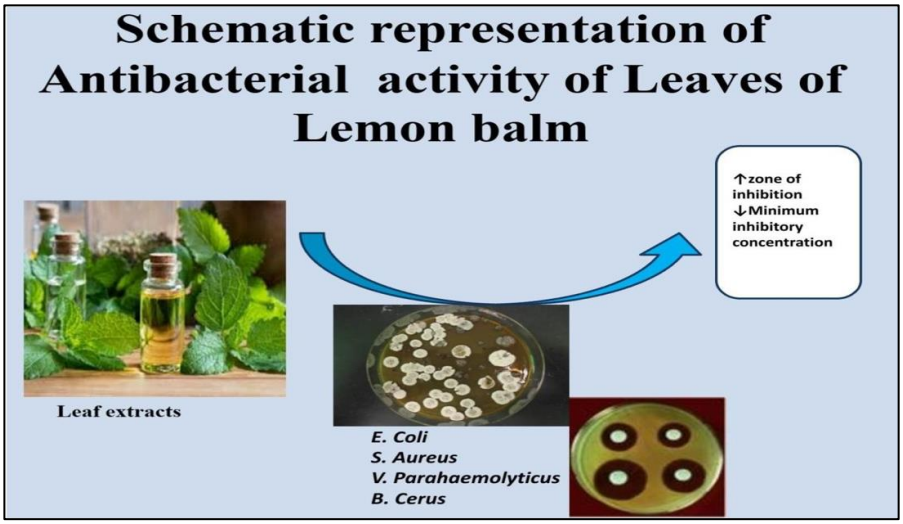

Figure 5.3: Schematic representation of Antibacterial activities of Leaves of Lemon balm in bacteria

5.7 Conclusion:

The aq. extract of lemon balm showed maximum microbial inhibition concentrations against *E. coli, S. aureus* also *Vibrio parahaemolyticus*. And they also exhibited maximum zone of inhibition in *S. aureus, E. coli* and *Vibrio parahaemolyticus.* The Ethanolic extract of lemon balm showed increased microbial inhibition concentration in *E. coli, S. aureus, Bacillus cereus* and also showed maximum microbial inhibition concentration in *E. coli, S. aureus, Bacillus cereus.* The maximal zone of inhibition was observed in *E. coli, S. aureus* and minimum concentration was in *Bacillus cereus*. The studies have confirmed that lemon balm exhibit antibacterial activity against a wide range of bacteria, it has been found to inhibit the growth of several bacteria responsible for common infections such as *Staphylococcus aureus, Escherichia coli.*

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6. Antimicrobial Activity of Mint (*Mentha Piperita*)

Darshini K. P., Surabhi M.

PG student,
Department of Food science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Manasa R.

Research Scholar,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Shekhara Naik R.

Professor and Head,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Mahesh Shivananjappa

Assistant Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Abstract:

Mentha piperita is a medicinal herb and strongly scented herb which belongs to family Lamiaceae, commonly known as mint. It has many pharmacological properties like antibacterial, antiviral, antifungal, antioxidant, anticancer and wound healing. The anti-microbial property is examined by extracting mint against different components such as essential oils, ethanol extract, hydro-alcoholic extract, menthol and menthone then testing it against few selected microorganisms like E. coli, s. aureus, pseudomonas aeruginosa, Enterobacter aerogenes, bacillus cereus, epidermidisa, Candida albicans. Several researches conducted on Mentha piperita combination of in vivo and in vitro techniques such as microdilution, agar diffusion, and bioautography. Experiment conducted on gram

positive and gram-negative microorganisms like B. cereus, S. aureus S. epidermidis, etc. shows minimal zone of inhibition against Mentha piperita L. essential oil extraction at concentration (1.0µg/ml). Candida albicans shows minimum zone of inhibition against hydro-alcoholic extract at concentration of (100mg/ml). E. coli and S. aureus showed minimal zone of inhibition against ethanol extract at concentration of (1µl/ml). C. albicans showed minimal zone of inhibition against ethanol extract at concentration of (0.125 µl/ml). This review aimed to explore the basic and clinical description of antimicrobial activity of Mentha piperita.

Keywords:

Essential oil, Menthol, Methyl acetate, Staphylococcus aureus, Staphylococcus epidermidis

6.1 Introduction:

The popular and generally used peppermint (*Mentha piperita*) which belongs to Lamiaceae family is a cultivated natural hybrid of *Mentha aquatic L.* (water mint). In spite of fact that a native genus of the Mediterranean region, it is cultured all over the world for its use in flavouring agent, medicinal, and pharmaceutical applications. Peppermint oil is one of the most popular produced and consumed essential oils (1). Human infections have increasing during the past 10 years, especially in immune-compromised patients. Among animal and human pathogens, dermatomycetes are the main cause of dermatomycoses (in hair, skin, and nails infections). Superficial infections are not life threatening, but chronic fungal infections of the epidermis transmit a considerable disease (2). The growth of microbial resistance to antibiotics is a global attention. Essential oils from the plants show more antibacterial activity from oxygenated terpenoids, phenols, alcoholic compounds, and other chemical constitutes that contribute to the antimicrobial effects (3). Microbial resistance is a growing problem, and the future of antimicrobial treatments remains undetermined. Multiple research institutions in Australia, Iran, Italy, Turkey, and elsewhere around the world have conducted studies on the antimicrobial properties of medical plants. Important research concerning the antimicrobial activity of plants has also been performed in Brazil (4). However, Antimicrobial activity of *Mentha piperita L.* against Candida albicans. in animals and humans. The estimated therapeutic benefits of medicinal plants are principally derived from subjective actual evidence gained from the practice of traditional medicine (5).

Figure 6.1: ***Mentha Piperita***

6.2 Morphological Description:

Mint is a perennial plant which grows up to 50–90 cm high, normally quadrangular and a prototypical member of the mint Family [6]. The branched stems are often purplish or tinged violet colour but sometimes they are gray-tomentose. The dark or light green leaves are short-petioled, with finely toothed margins. The flowers are purple or pinkish in colour which having false spikes and they rarely bear seeds.

Mint is generally sterile and spreads by means of runners. The plant usually grows in a sunny side and it prefers acid, neutral and basic, light, medium soils but can also grow in heavy clay soil [7].

6.3 Traditional Uses:

The essential oil of mint is used in Western and Eastern traditional drug as a remedy against anti-spasmodic, anti-septic, aromatic and also for treatment of colds, nausea, sore throat, cancers, toothaches, cramps and indigestion. MP is reported for its therapeutic use in Chinese traditional medicine. It is used as a carminative, stimulant, tonic, anti-viral and anti-fungal agent [8]. The scientific studies provide awareness on the use of peppermint for biological effects such as anti-oxidant, anti-microbial, anti-viral, anti-inflammatory, biopesticidal, larvicidal, anticancer, radioprotective effect, respiratory problems, gastrointestinal/hepatic disorders, genotoxicity and anti-diabetic activity [9].

IV Pharmacological studies:

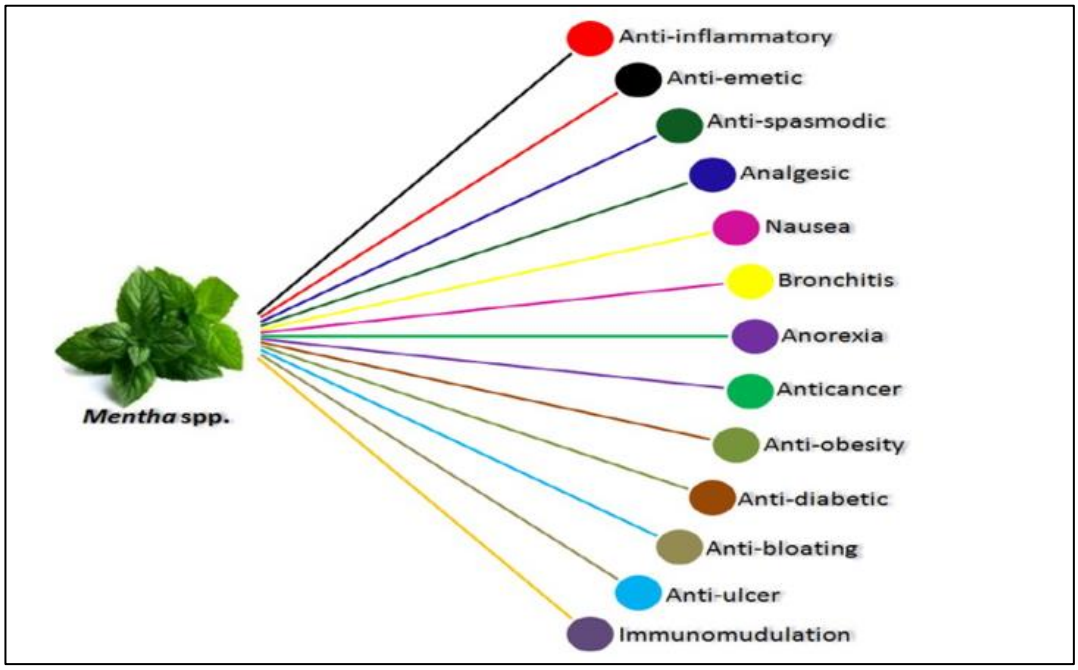

Figure 6.2: pharmacological studies of MP

Pharmacological studies indicated that mint had been used extensively as medicinal plant in health care for centuries. The mint and its elements possess several pharmacological actions [10].

It has many pharmacological properties like antibacterial, antiviral, antifungal, antioxidant, anticancer and wound healing. Antimicrobial is an agent that kills microorganisms or stops their growth such as bacteria, fungi, or protozoan's.

The anti-microbial property is examined by extracting mint against different components such as essential oils, ethanol extract, hydroalcoholic extract, menthol and menthone then testing it against few selected microorganisms like *E. coli, s.aureus, pseudomonas aeruginosa, Enterobacter aerogenes, bacillus cereus, s.epidermidis, Candida albicans.* Several researches conducted on Mentha piperita combination of in vivo and in vitro techniques such as microdilution, agar diffusion, and bioautography.

6.5 Antimicrobial Activity of Mint:

Heywood, V. h in 1979 studied the antimicrobial activity on MP. here while studying the antimicrobial activity on *E.coli* as treatment they used menthol 1.25 mg/mL and menthone extraction 5.0 mg/mL where as a result they observed (2.5-1.25 mg/mL) zone of inhibition and minimum inhibitory concentration 1µl/ml where as a result they observed (0.0035) zone of inhibition . In the same antimicrobial activity menthol 36.02 % and menthone extraction 24.56 % where observed (27.02 mm) zone of inhibition [11].

J. agric. Food chem in 2002 studied the antimicrobial activity on *staphylococcus aureus* as treatment they used menthol 0.625 mg/mL and menthone extraction 2.5 mg/mL where as a result they observed (0.625 mg/mL) zone of inhibition and minimum inhibition concentration 1µl/ml the zone of inhibition(0.125mm) is observed [12].

J. agric. Food chem in 2002 studied the antimicrobial activity on *pseudomonas aeruginosa* as treatment they used menthol 2.5 mg/mL and menthone extraction 2.5 mg/mL where as a result they observed (2.5 mg/mL) zone of inhibition [12].

J. agric. Food chem in 2002 studied the antimicrobial activity on *Enterobacter aerogenes* as treatment they used menthol 1.25 mg/mL and menthone extraction 5.0 mg/mL where as a result they observed (1.25 mg/mL) zone of inhibition [12].

J. agric. Food chem in 2002 studied the antimicrobial activity on *proteus vulgaris* as treatment they used menthol 1.25 mg/mL and menthone extraction 2.5 mg/mL where as a result they observed (2.25 mg/mL) zone of inhibition [12].

Adams, r. p in 2001 studied the antimicrobial activity on *salmonella typhimurium* as treatment they used menthol 0.625 mg/mL and menthone extraction 5.0mg/mL where as a result they observed (1.25mg/mL) zone of inhibition. In same antimicrobial activity treatment, they used menthol 36.02% and menthone extraction 24.56% the zone of inhibition is (20.06mm) is observed [13].

Adams, r. p in 2001 studied the antimicrobial activity on *Klebsiella pneumoniae* as treatment they used menthol 2.5 mg/mL and menthone extraction 5.0mg/mL where as a result they observed (2.5mg/mL) zone of inhibition. In same antimicrobial activity treatment, they used menthol 36.02% and menthone extraction 24.56% the zone of inhibition is (14.24mm) is observed [13].

J. agric. Food chem in 2002 studied the antimicrobial activity on *Yersinia enterocolitica* as treatment they used menthol 2.5 mg/mL and menthone extraction 2.5 mg/mL where as a result they observed (2.5 mg/mL) zone of inhibition [12].

J. agric. Food chem in 2002 studied the antimicrobial activity on *Listeria monocytogenes* as treatment they used menthol 0.625 mg/mL and menthone extraction 2.5 mg/mL where as a result they observed (0.156-0.625 mg/mL) zone of inhibition [12].

J. agric. Food chem in 2002 studied the antimicrobial activity on *bacillus cereus* as treatment they used menthol 1.25 mg/mL and menthone extraction 1.25 mg/mL where as a result they observed (1.25 mg/mL) zone of inhibition [12].

J. agric. Food chem in 2002 studied the antimicrobial activity on *staphylococcus epidermidis* as treatment they used menthol 0.625 mg/mL and menthone extraction 0.625 mg/mL where as a result they observed (0.625 mg/mL) zone of inhibition [12].

J. agric. Food chem in 2002 studied the antimicrobial activity on *Xanthomonas campestris pv. phoseoli* as treatment they used menthol 0.625 mg/mL and menthone extraction 1.25 mg/mL where as a result they observed (0.07-1.25 mg/mL) zone of inhibition [12].

J. agric. Food chem in 2002 studied the antimicrobial activity on *Pseudomonas syringae pv. phaseolicola* as treatment they used menthol 1.25 mg/mL and menthone extraction 2.5 mg/mL where as a result they observed (0.07-1.25 mg/mL) zone of inhibition. In same antimicrobial activity treatment they used menthol 0.07mg/mL and menthone extraction 1.25mg/mL the zone of inhibition (0.07-1.25 mg/mL) is observed [12].

J. agric. Food chem in 2002 studied the antimicrobial activity on *Pseudomonas syringae pv. tomato* as treatment they used menthol 0.156 mg/mL and menthone extraction 2.5 mg/mL where as a result they observed (0.07-1.25 mg/mL) zone of inhibition [12].

Adams, r. p in 2007 studied the antimicrobial activity on *Xanthomonas campestris pv. campestris* as treatment they used menthol 0.156 mg/mL and menthone extraction 1.25mg/mL where as a result they observed (0.07-1.25mg/mL) zone of inhibition. In same antimicrobial activity treatment, they used menthol 36.02% and menthone extraction 24.56% the zone of inhibition (3.18mm) is observed [13].

Cox sd, mann CM, markham JL in 2000 studied the antimicrobial activity on *Candida albicans* as treatment they used menthol 0.625mg/mL and menthone extraction 2.5 mg/mL the zone of inhibition (2.5mg/mL) is observed the MIC 0.125µl/ml the zone of inhibition (0.028mm) observed. In the same antimicrobial activity treatment, they used HALEX 100mg/mL the zone of inhibition (5 and 10mm) observed [14].

J. agric. Food chem in 2002 studied the antimicrobial activity on *B. cereus* as treatment they used menthol 36.02 % and menthone extraction 24.56 % where as a result they observed (32.08mm) zone of inhibition [11].

Marina d. in 2006 studied the male wistar rats on antifungal activity 2 month old wistar rats as treatment they used 1% solution of essential oil and menthol extraction as given for once a day during the 36 days the zone of inhibition (0.32-0.2mm) observed [15].

Table 6.1: Antimicrobial Activity of Mint

Sr. No.	Model	Treatment	Results (Zone of inhibition)	Reference
1	*E. coli*	• M1 1.25 mg/m • L • M2 5.0 mg/mL • MIC 1µl/ml • M1 (36.02%) • M2 (24.56%)	2.5-1.25 mg/mL 0.0035 mm 27.02 mm	[11]
2	*Staphylococcus aureus*	• M1 0.625 mg/mL • M2 2.5 mg/mL • MIC (1µl/ml)	0.625 mg/mL 0.125 mm	[12]
3	*Pseudomonas aeruginosa*	• M1 2.5 mg/mL • M2 5.0 mg/mL	2.5 mg/mL	[12]
4	*Enterobacter aerogenes*	• M1 1.25 mg/mL • M2 5.0 mg/mL	1.25 mg/mL	[12]
5	*Proteus vulgaris*	• M1 1.25 mg/mL • M2 2.5 mg/mL	2.25 mg/mL	[12]
6	*Salmonella typhimurium*	• M1 0.625 mg/mL • M2 5.0 mg/mL • M1 (36.02%) • M2 (24.56%)	1.25 mg/mL 20.06 mm	[13]
7	*Klebsiella pneumoniae*	• M1 2.5 mg/mL • M2 5.0 mg/mL • M1 (36.02%) • M2 (24.56%)	2.5 mg/mL 14.24 mm	[13]

Sr. No.	Model	Treatment	Results (Zone of inhibition)	Reference
8	*Yersinia enterocolitica*	• M1 2.5 mg/mL • M2 2.5 mg/mL	2.25 mg/mL	[12]
9	*Listeria monocytogenes*	• M1 0.625 mg/mL • M2 2.5 mg/mL	0.156-0.625 mg/mL	[12]
10	*Bacillus cereus*	• M1 1.25 mg/mL • M2 1.25 mg/mL	1.25 mg/mL	[12]
11	*Staphylococcus epidermidis*	• M1 0.625 mg/mL • M2 0.625 mg/mL	0.625-2.5 mg/mL	[12]
12	*Xanthomonas campestris pv . phaseoli*	• M1 0.625 mg/mL • M2 (1.25 mg/mL)	0.07-1.25 mg/mL	[12]
13	*Pseudomonas syringae pv. phaseolicola*	• M1 (1.25 mg/mL) • M2 (2.5 mg/mL)	0.07-1.25 mg/mL	[12]
14	*Pseudomonas syringae pv. phaseolicola*	• M1(0.07 mg/mL) • M2 (1.25 mg/mL)	0.07-1.25 mg/mL	[12]
15	*Pseudomonas syringae pv. tomato*	• M1(0.156 mg/mL) • M2(2.5 mg/mL)	0.07-1.25 mg/mL	[12]
16	*Xanthomonas campestris pv. campestris*	• M1(0.156 mg/mL) • M2(1.25 mg/mL) • M1 (36.02%) • M2 (24.56%)	0.07-1.25 mg/mL 3.18 mm	[13]
17	*Candida albicans*	• M1 (0.625 mg/mL) • M2(2.5 mg/mL) • HALEX (100 mg/mL) • MIC (0.125 µl/ml)	2.5 mg/mL 5 and 10 mm 0.028 mm	[14]
18	*B. cereus*	• M1 (36.02%) • M2 (24.56%)	32.08 mm	[12]
19	♂ wistar rats on antifungal activity 2-month-old wistar rats	1% solution of essential oil and menthol as given for once a day during the 36 days.	0.32 mm-0.2 mm	[15]

6.6 Conclusion:

MP is a traditional plant commonly known as pippermint. It belongs to the family Lamiaceae. It is a strongly scented herb which grows in the temperate regions. Mint has both medicinal as well as commercial importance. Mint has many pharmacological properties which promotes health. It has enormous medicinal values in the traditional uses. It is commonly known as pudina in India. The studies have shown that the application of the ethyl, menthol and ethanol extract of mint has inhibited the growth of both Gram-positive and Gram-negative bacteria, thus peppermint oil can be used as a good preserving agent for inhibiting some microorganisms.

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7. Optimization of Fermentation Time and Its Effect on The Organoleptic Properties of Bhatura from Sorghum (*Sorghum bicolour*)

Deepika M.

PG student,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Manasa R.

Research Scholar,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Shekhara Naik R.

Professor and Head,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Mahesh Shivananjappa

Assistant Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Abstract:

Fermentation is the traditional food processing technique which microbial activity increases the bio-accessibility of nutrients and enhances the texture of food products. Bhatura is a deep fried, leavened sourdough bread which has a unique flavour profile due to lactic acid fermentation mainly by lactic acid bacteria (LAB). Sorghum is the most commonly used millet & it is a rich source of dietary fibre, protein, iron and other micronutrients including phenolic compounds and antioxidants. The study was carried by

preparing Bhatura in six formulations from refined wheat flour replaced with sorghum flour in different proportions. The subjective evaluation of Bhatura resulted with high acceptance for SB3 (40%) variation& it was subjected to optimize the lactic acid fermentation time. On its organoleptic evaluation, fermentation for three hours acquired high acceptance. The proximate analysis of nutrients in selected variation had increased Protein, fibre, Iron & Phosphorus levels when compared to standard. Moreover, it also reduced the carbohydrate content of Bhatura making it low glycaemic in nature.

Keywords:

jowar, low-glycaemic, gramaeceae, lactobacillus, hetero-fermentation

7.1 Introduction:

Fermentation is a biochemical process used to obtain energy (ATP) through the partial oxidation of sugars, with the release of acids and alcohols as intermediate products, facilitated by microorganisms. Fermented foods generally preserve pleasant flavour, aroma, texture, enhanced nutritive values, and good keeping quality under ambient conditions (1). Lactobacillus species, including Streptococcus thermophiles, Lactococcus lactis, and Lactobacillus and Bifidobacterium, are the principal microorganisms responsible for fermentation. Lactic acid bacteria are known to prevent the growth of pathogens, degrade mycotoxins, and have probiotic capabilities (2). Fermentation provides authentic sensory characteristics to the product and also increases the bioavailability of nutrients by reducing anti-nutritional factors. Curd, a probiotic-rich dairy product, acts as a live inoculum to aid in fermentation (3). The level of fermentation varies with the type of ingredients, time, temperature, inoculum, substrate concentration, etc. Sorghum is the fifth largest produced millet in the world. It is an exceptional source of nutrients and bioactive components, contributing as a staple diet. Sorghum millet contains moisture content (52%), energy (193 kcal), carbohydrates (39.8g), protein (7.1g), fat (0.6g), fibre (0.9g), calcium (10mg), iron (3.5mg), and niacin (1.7mg). It also contains phytochemicals such as phenols, polyflavonols, thiols, anthocyanins, tannins, 3-deoxyanthocyanidin, flavone, and flavanone (4). The majorly found phenolic acid in sorghum millet is 3-deoxyanthocyanidins (6) (7). Sorghum millet, termed as 'The king of millets,' is gluten-free like other millets and can exhibit a protective effect against cardiovascular disease (CVD), obesity, diabetes, hypercholesterolemia, and constipation. Incorporating jowar in the diet can bring noticeable improvements to an individual's health. Bhatura is a North Indian fermented product that resembles puri. It is made with refined wheat flour as the base ingredient. Due to partial fermentation, it has an elastic and chewy texture. Partial replacement of sorghum flour in different concentrations increases nutrient levels and enhances sensory attributes of the product (9).

7.2 Objective:

- To develop Millet based fermented product and evaluate its organoleptic properties
- To optimize the fermentation time of the developed product
- To obtain low gluten and low glycaemic food

7.3 Materials and Methods:

- **Raw materials**: The study was carried out in the Department of food science and nutrition, Yuvaraja's college, (Autonomous) University of Mysore, Mysuru. The raw materials viz., refined wheat flour, semolina, baking soda, baking powder, sugar, curd, oil and salt were purchased from local grocery shop of Mysuru, Karnataka, India.
- **Methods:** Bhatura was prepared by mixing sorghum flour the ingredients except curd, oil, & water. Add curd and required amount of water to make semi- hard dough. The dough was tucked and smeared with oil and it was left to rest for 2 hours. The partially fermented dough was made into equal sized balls and flattened into sheets of uniform thickness. Tuck the dough and smear oil over its surface. Close the bowl with a muslin cloth and leave it to rest for 2 hours. Make equal sized balls and flatten them to obtain uniform thickness. Then it was deep-fried till the product was golden brown and crisp.

7.4 Optimization of Fermentation Time:

The fermentation of Bhatura was determined in most acceptable variation (40%) by preparing dough by following the standard procedure and its fermentation time was varied as 1, 2, 3, & 4 hours respectively at room temperature. The Bhatura was prepared out of these variations.

7.4.1 Determination of 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 using the pH paper. The dough was washed with distilled water and pH paper was dipped in that water for pH analysis.

7.4.2 Sensory Evaluation:

Bhatura was developed and evaluated for its organoleptic properties [colour, appearance, flavour, texture, taste and overall acceptability] keeping standard Bhatura as reference. It was carried out by 30 semi-trained panellists. Hedonic scale (1- 9 Ratings) was used for rating the sensory quality of the sorghum Bhatura. The mean value of 30 score cards was considered for evaluating the sensory quality.

7.4.3 Proximate Analysis:

The proximate estimation was carried out by following standard AOAC (1990) method for chosen variation SMD3 (40%) & control (10). These methods had good accuracy & precision. The moisture content was evaluated using hot air oven at 98-100 degree Celsius (11), whereas protein content was estimated using standard Micro-Kjeldhal method by determining total nitrogen content (12), 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 (10). Phosphorous and iron was estimated by inductively Coupled Plasma Mass Spectrometry (ICPMS) (13)(14).

7.4.4 Statistical Analysis:

Each sample was analysed in triplicates. The data obtained was analysed statistically using standard methods given by Snedecor and Cochran (15) and by Duncan's multiple range test with the $p \leq 0.05$ consider to be significant (16).

Table 7.1: Formulation of The Products (Ingredients G/100g) For Preparation of Sorghum Millet Bhatura

Sr. No.	Ingredients (g)	0%	20%	40%	60%	80%	100%
1	Maida flour	100	80	60	40	20	-
2	Sorghum flour	-	20	40	60	80	100
3	Fine semolina	15	15	15	15	15	15
4	Curd (ml)	30	30	30	30	30	30
5	Salt	2	2	2	2	2	2
6	Baking powder	1.25	1 25	1.25	1.25	1.25	1.25
7	Baking soda	1.25	1.25	1.25	1.25	1.25	1.25
8	Sugar	2.5	2.5	2.5	2.5	2.5	2.5
9	Oil	10	10	10	10	10	10
10	Water	40	40	45	50	55	65

7.5 Formulation of the Product:

Take a bowl and mix sorghum flour and other ingredients in a bowl except, curd, oil and water

↓

Add hot oil to it and mix it again

↓

Add curd and required amount of water to make semi- hard dough

↓

Tuck the dough and smear oil over its surface

↓

Close the bowl with a muslin cloth and leave it to rest for 2 hours

↓

Make equal sized balls and flatten them to obtain uniform thickness

↓

Deep-fry them till it become golden brown & crisp

Figure 7.1: Flow chart for preparation of Sorghum Bhatura (10)

Figure 7.2: Bhatura with Different Proportions of Sorghum

Figure 7.3: Sorghum Bhatura (40%) With Variable Fermentation Time

A. Determination of pH in dough of sorghum Bhatura fermented for variable duration.

Table 7.2: pH of Dough Raised Due to Fermentation.

Variations	Initial pH	pH after 1 hrs	pH after 1 hrs	pH after 1 hrs	pH after 1 hrs
Standard	6	5	5	5	5
SMD 20%	6	5	5	5	5
SMD 40%	6	5	5	5	5
SMD 60%	6	5	5	5	5
SMD 80%	6	5	5	5	5
SMD 100%	6	5	5	5	5

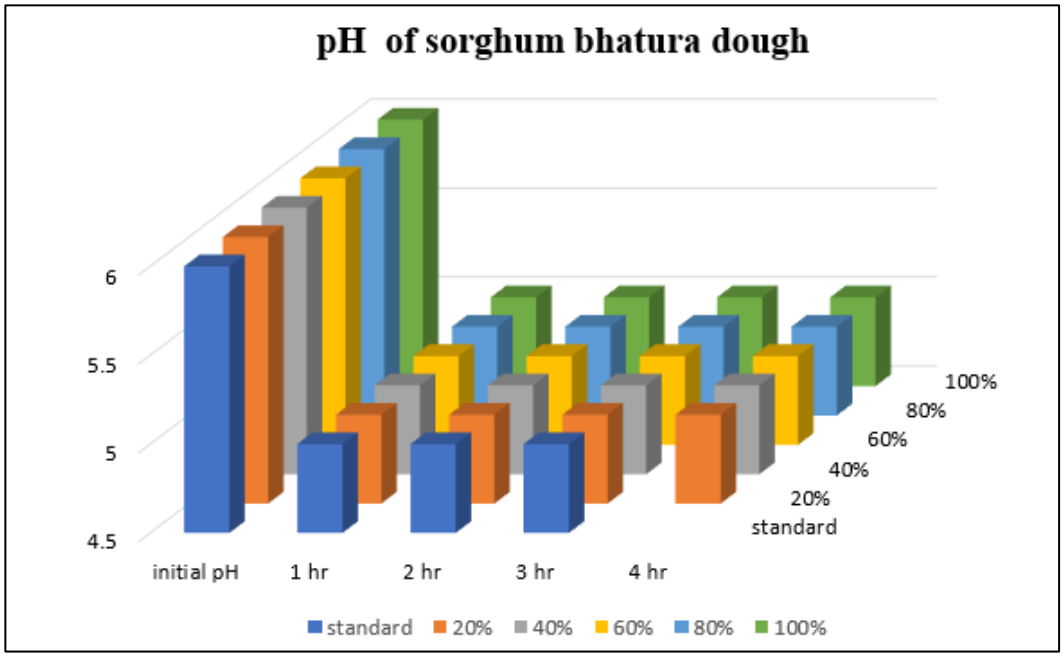

Figure 7.3: Ph of Sorghum Bhatura Dough

7.6 Sensory Analysis of Sorghum Incorporated Bhatura:

Table 7.3: sensory scores of Bhatura prepared by varying the fermentation time of Sorghum millet & refined wheat flour.

parameters	Standard RWF1	SB2 (20%)	SB3 (40%)	SB4 (60%)	SB5 (80%)	SB6 (100%)
Appearance	8.81±0.39	8.26±0.79	8.51±0.56*	7.6±0.45	7.2±0.56	6.53±0.63
color	8.72±0.45	8.26±0.88	8.26±0.79	7.98±0.23	7.23±0.79	6.53±0.19
texture	8.72±0.45	8.20±0.77	8.86±0.51	7.81±0.25	6.86±0.83	6.46±0.77
taste	8.68±0.56	8.21±0.86	8.63±0.45	7.4±0.70	7.01±0.65	6.82±0.51
flavor	8.68±0.56	8.13±0.83	8.53±0.65	7.91±1.12*	7.2±0.10	6.73±0.91
overall acceptability	8.72±0.45	8.26±0.59	8.71±0.52	7.86±0.91	7.3±0.25	6.61±0.05*

Values are mean ± SD (n=30) *p value < 0.05 (Holm sidak method)

A. Sensory analysis of Bhatura made from varying the fermentation time:

Table 7.4: sensory scores of Bhatura prepared by varying the fermentation time of Sorghum millet & refined wheat flour.

Parameters	FSB1(1hr)	FSB2(2hr)	FSB3 (3hr)	FSB4(4hr)
Appearance	7.81±0.36	7.9±0.96	8.6± 0.51*	7.5±0.73
Color	8.01±0.15	8.0±0.48	8.3±0.52*	8.1±0.42
Texture	7.32±0.85	7.8±0.42	8.6±0.45*	7.2±0.24
Taste	7.10±0.23	8.1±0.87	8.7±0.40*	7.1±0.24
Flavor	7.01±0.62	7.9±0.51	8.5±0.52 *	7.0±0.46
Overall acceptability	7.26±0.54	8.0±0.42	8.8±0.42*	7.0±0.72

Values are mean ± SD (n=30) *p value < 0.05 (Holm sidak method)

B. Proximate Analysis of Nutritional Composition of Sorghum Incorporated Bhatura:

Table 7.5: Proximate Nutritional Composition of Sorghum Based Bhatura (40%)

Nutrients	Standard Bhatura (RWF)	SMB3
Moisture (%)	2.2±0.16	2.5±0.09
Carbohydrate (g)	60.73±0.45	58.55±0.12
Protein (g)	11.7±0.37	13.25±0.19*
Fat (g)	22.9±0.96	23.1±0.34*
Crude Fiber (g)	0.2±0.31	0.8±0.11*
Ash (g)	2.2±0.26	1.8±0.13
Energy (kcal)	495.82±0.12	495.1±0.10
Iron (mg)	7.5±0.50	9.02±0.36*
Phosphorus(mg)	164.98±0.18	206.38±0.41*

Values are mean ± SD (n=3) *p value < 0.05 (Holm sidak method)

7.7 Results and Discussion:

Sensory analysis of Sorghum incorporated Bhatura:

The development of Bhatura using different formulations resulted in the acceptance of the 40% variation based on sensory evaluation. This acceptance was due to reduced ratings in appearance, texture, color, and flavor. Refined wheat flour (Maida) is rich in gluten and gliadin proteins, while sorghum is gluten-free.

As the concentration of sorghum increased in the Bhatura dough, the viscoelasticity of gluten and gliadin decreased simultaneously. This affected the chewy, soft texture of Bhatura, making it slightly harder in nature.

Optimization of Fermentation Time and Determination of Ph of The Dough:

The acceptance of the 3-hour fermented product was observed during optimization. This indicates that the fermentation time may vary depending on the type and proportion of ingredients, which can impact the organoleptic properties of Bhatura. Acidification is the main factor contributing to the authentic sourness in fermented products. pH is a parameter used to determine the level of acidification in products, and it can vary depending on various factors, including the proportion of ingredients used in product development. Therefore, as the fermentation time increased, a reduction in pH was observed.

Proximate Nutritional composition of Sorghum bhatura : The developed product was found to be Enriched with certain nutrients when compared to the standard bhatura as reference. It was composed of significant increased levels of protein, Crude fibre, Iron and phosphorous. Slightest reduction in carbohydrate levels was found in sorghum bhatura.

7.8 Conclusion:

The present study was an attempt to develop a value-added product with the incorporation of sorghum, a millet that is affordable and rich in nutrients with lower levels of gluten. The 40% partial replacement of Maida with sorghum flour based Bhatura was the most acceptable one when compared with standard as per sensory parameters. Fermentation period of 3 hours was found to be apt for the selected variation (40%). The optimized fermentation and sorghum incorporation resulted in enhanced nutritional profile. Increased levels of dietary fiber, protein, Iron was observed. The significantly reduced levels of carbohydrate substantiated the developed product as low - glycemic Food. pH reduced with increase in fermentation time. Whereas the dough volume increased simultaneously with period of fermentation due to leavening. Probiotic rich curd aids lactic acid fermentation that increases gut micro flora in the body. Therefore, Bhatura as a popular dish with touch of new formulation with sorghum flour and optimized fermentation can be considered as best choice over a normal Product.

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8. Effect of Fermentation Time on Sensory Attributes of Little Millet (*Panicum Sumatrense*) Dosa

Gagana N. K.

PG student,
Department Of Food Science and Nutrition,
Yuvaraja's College
(Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Manasa R.

Research Scholar,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Shekhara Naik R.

Professor and Head,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Mahesh Shivananjappa

Assistant Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Abstract:

Fermentation is a natural process through which microorganisms like yeast and bacteria convert carbs — such as starch and sugar — into alcohol or acids. Fermented foods, like dosa, contain beneficial bacteria called probiotics. These probiotics can help improve gut health by promoting the growth of beneficial gut bacteria. Dosas are popular in South Asia as well as around the world. Dosas are served hot, often with chutney and sambar.

Little millet is rich in Magnesium which helps improve heart health. It is also rich in Niacin which helps lower cholesterol. Little millet contains phosphorus which is great for weight loss, tissue repair and energy production. This study was conducted to develop and evaluate fermented dosa of six different compositions L1, L2, L3, L4, L5 and L6 containing rice, black gram dhal, fenugreek with different proportions of little millet (0%, 20%, 40%, 60%, 80%, 100%) we were developed and evaluated for acceptability using subjective and objective method by taking rice dosa as a control.

These formulations were analysed for sensory attributes [n=20] and the sensory score is highest for L4 and its nutrient composition was recorded. Then fermentation time was varied in L4 formulation for 6 hours, 12 hours, 18 hours, 24 hours for evaluation of fermentation quality. The dosa prepared with 6 hours fermentation time was more acceptable. Proximate nutritional composition of the best accepted varition were analysed by standard procedure. Dosa prepared from little millet had more protein, fibre, calcium and phosphorus. They are gluten free and have low glycemic index compared to traditional dosa, since they contain less carbohydrates and more fibre.

Keywords:

Fermentation, probiotis , glycemic index, dietary fibre.

8.1 Introduction:

Millets are one among the cereals apart from the rice, maize and wheat. 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].

Dosa is a thin pancake in South Indian cuisine made from a fermente5d batter of ground black lentils and rice. Dosas are popular in South Asia as well as around the world [4]. Dosas are served hot, often with chutney and sambar. Dosa is high in carbohydrates and contains no added sugars or saturated fats.

As its key ingredients are rice and black gram, it is a good source of protein. A typical homemade plain dosa without oil contains about 112 calories, of which 84% is carbohydrate and 16% protein. The fermentation process increases the vitamin B and vitamin C content [5].

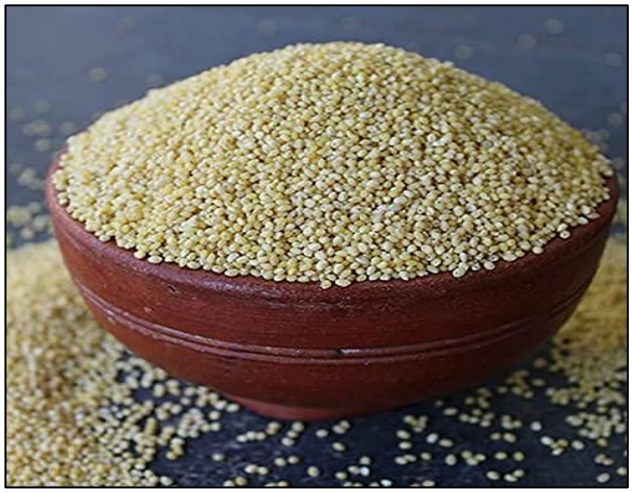

Figure 8.1: Millets

8.2 Objective:

- To develop little millet-based Dosa by partially replacing parboiled rice with little millet.
- To evaluate organoleptic acceptability of the developed product.
- To study the effect of fermentation time on the developed product

8.3 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, fenugreek seeds and salt were produced from the local market of Mysuru.
- **Method of preparation:** Dosa was prepared by soaking different ratio of parboiled rice, black gram dal, fenugreek seeds 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. Heat dosa pan and pour ladle full of batter and cook both the sides by add 2-3 drops of oil.
- **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.
- **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, 18 and 24 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.
- **Sensory Analysis of Prepared Dosa:** 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 average score of the 20 semi trained panellists by using 9-point hedonic scale.

- **Nutritional analysis of prepared Dosa:** Standard AOAC (1980) method was used to determine the nutritional composition of selected variation (LM4) of little 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- 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 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 [6].
- **statistical analysis:** Each sample was analysed in triplicates. The data obtained was analysed statistically using standard methods given by Snedecor - and Cochran [8] and by Duncan's multiple range test with the $p \leq 0.05$ consider to be significant [9].

8.4 Formulation of the Product:

Ingredients	**LM1 (0%)**	**LM2 (20%)**	**LM3 (40%)**	**LM4 (60%)**	**LM5 (80%)**	**LM6 (100%)**
Parboiled rice (g)	100	80	60	40	20	0
Little millet (g)	0	20	40	60	80	100
Black gram dhal (g)	25	25	25	25	25	25
Fenugreek (g)	5	5	5	5	5	5

Wash and soak, rice, black gram dhal, fenugreek seeds and little millet for 4hrs

↓

Add soaked rice and little millet in a jar and grind it.

↓

Add water as needed, grind untill smooth batter is obtained

↓

Keep it overnight (8-12hrs) for fermentation.

↓

Heat dosa pan and pour ladle full of batter

↓

Cook both the sides by add 2-3 drops of oil

Flow chart for the preparation of little millet Dosa

Figure 8.2: Standard Dosa and Dosa's prepared by incorporation of LM

8.5 Result and Discussion:

8.5.1 Sensory Evaluation of Little Millet Dosa:

The study was undertaken to prepare millet-based Dosa by partially replacing parboiled rice with little millet. The data pertaining to the effect of incorporation of various levels of LMD (20, 40, 60, 80 and 100%) on sensory attributes of Dosa and the results are shown in Table 8.2. The scores obtained for all sensory attributes for LM2, LM3, LM4, were similar to the control, whereas LM5 and LM6 showed decreased score and were less acceptable compared to the other variations. The acceptable LM3 variation was incubated for different fermentation time to study the optimum fermentation time of little millet incorporated Dosa.

Table 8.2: sensory evaluation of different variation of dosa developed from little millet with partial replacement of rice. Values are mean ± SD, p ≤ 0.005 (Holm Sidak method), n =20.

Variation	L1 (0%)	L2 (20%)	L3 (40%)	L4 (60%)	L5 (80%)	L5 (100%)
Appearance	9±0	7.8±0	7±0	7.9±0.3	6.8±0.5	6.1±0.4*
Color	9±0	7.7±0.3	7±0	7.8±0.2	6.8±0.5	5.9±0.2*
Texture	8.9±0.2	7.6±0.3	7±0	7.8±0.2	6.3±0.2	6±0*
Taste	9±0	7.6±0.2	6.6±0.1	7.8±0.2	6.4±0.3	6.1±0.2*
Flavor	9±0	7.7±0.3	6.1±0.2	7.9±0.6	6.6±0.3	6±0*
Overall acceptability	9±0	7.8±0.3	6.7±0.4	7.9±0.7	6.5±0.4	5.9±0.6*

8.5.2 pH:

pH value of Dosa 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.

Table 8.3: pH of Dosa batter

Variation	Initial pH	pH after 6 hrs	pH after 12 hrs	pH after 18 hrs	pH after 24 hrs
LM (control)	6	5	5	5	5
LM (20%)	6	5	5	5	5
LM (40%)	6	5	5	5	5
LM (60%)	6	5	5	5	5
LM (80%)	6	5	5	5	5
LM (100%)	6	5	5	5	5

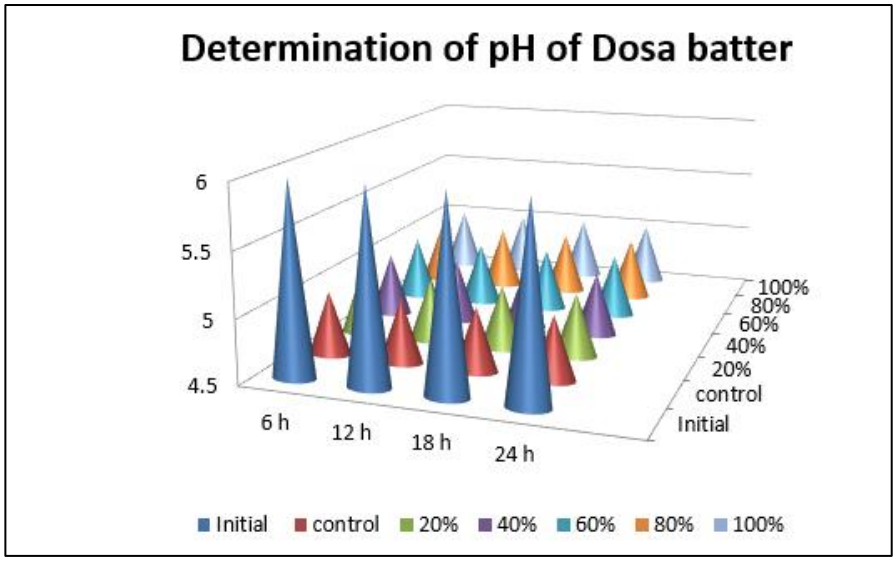

Figure 8.3: pH of Dosa batter

8.5.3 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 Dosa batter is due to increase incorporation of the lactic acid bacteria into the batter during fermentation and entrapment of air.

Table 8.4: Determination of Volume of Dosa Batter

Variation	Initial volume(ml)	Volume after 6 hrs (ml)	Volume after 12 hrs (ml)	Volume after 18 hrs (ml)	Volume after 24 hrs (ml)
LM (control)	40	50	55	59	52
LM (20%)	40	50	54	56	53
LM (40%)	40	49	50	56	46
LM (60%)	40	56	65	72	78
LM (80%)	40	45	51	56	45
LM (100%)	40	40	40	40	40

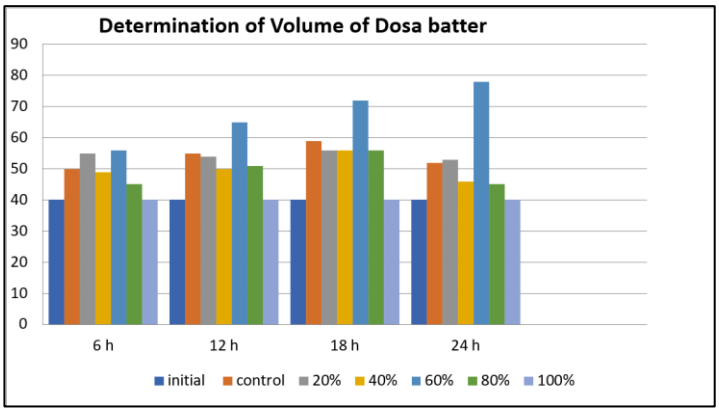

Figure 8-4: Volume of Dosa batter

8.5.4 Optimization of Batter Fermentation

The data pertaining to the effect of incubation of different fermentation time of little millet grains (6hrs, 12hrs, 18hrs, 24hrs). The sensory attributes of Dosa and the results are shown in Table. The scores obtained for all sensory attributes of LM1, LM2, LM3 and LM4. The variation LM1 was more acceptable in terms of sensory attributes.

Table 8.5: sensory evaluation of acceptable dosa developed from little millet with partial replacement of rice incubated at different fermentation time.

Fermentation time	LM (6 hour's)	LM (12 hour's)	LM (18 hour's)	LM (24 hour's)
Appearance	**8.1±0.3**	7.9±0.2	7.1±0.1	6.3±0.4*
Color	**8.2±0.1**	7.4±0.5	6.6±0.6	6.8±0.5*
Texture	**8.4±0.2**	7.2±0.4	6.3±0.7	6.4±0.3*
Taste	**8.4±0.2**	7.3±0.5	6.4±0.4	5.4±0.2*
Flavor	**8.3±0.4**	7.6±0.7	5.0±0	6.4±0 5*
Overall acceptability	**8.3±0.4**	7.4±0.6	6.7±0.4	6.4±0.5*

Values are mean ± SD, $p \leq 0.005$ (Holm Sidak method), n = 20.

Figure 8.5: Little Millet Incorporated Dosa Developed by Varying Fermentation time

8.5.5 Nutritional Analysis of Prepared Dosa LM4 (60%):

The proximate composition of acceptable little millet dosa (LM4) and that of control were analysed and the results of the same are shown in Table 4. The values of moisture, protein, dietary fiber and calcium content was higher in LM4 than that of control, whereas carbohydrate was less. However, ash, iron and phosphorous content were increased in little millet dosa.

Table 8.6: proximate composition of selected variation [LM (60%)] of dosa developed from little millet with replacement of rice.

Nutrients	Standard	LM (60%)
Moisture (%)	20.05±0.15	24.4±0.25*
Carbohydrates (g)	57.7±1.08	51.40±0.45
Protein (g)	18.82±0.03	19.77±0.48*
Fat (g)	1.04±0.25	1.76±0.35*
Crude fibre (g)	0.74±0.01	0.98±0.05*
Ash (g)	1.65±0.37	1.70±0.04*
Energy (kcal)	315.44±0.27	395±0.15*
Iron (mg)	3.69±0.02	6.2±0.04*
Phosphorus (g)	257.75±0.05	272.2±0.53*

Values are mean ± SD, $p \leq 0.005$ (Holm sidak), n = 3

8.6 Conclusion:

Dosa is a type of pan cake, originating from South India, popular as a breakfast food in Southern India. Here we have attempted to develop dosa 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 dosa batter. The two species *L. mesenteroides* and *S. faecalis* mainly present the fermented batter of dosa. *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 LM1 (6h). Little millet dosa of 60% (LM4) had highest acceptability in terms of sensory scores where as other variations had least acceptability. Little millet dosa 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 dosa was found to be 6h and acceptable upto 60%.

8.7 References:

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9. Standardization of Fermentation Time and Quality Evaluation of Punugulu from Sorghum (*Sorghum Bicolor*)

Kavya M. R.

PG student,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Manasa R.

Research Scholar,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Shekhara Naik R.

Professor and Head,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Mahesh Shivananjappa

Assistant Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Abstract:

Fermentation is the metabolic process in which starch or sugar convert into alcohol or an acid anaerobically releasing energy. It helps to increase digestion and bioavailability of nutrients, as well as improve the texture and taste of the product. Punugulu is a traditional fermented deep-fried snack from Andhra Pradesh. This research includes the development and quality evaluation of Punugulu by replacing rice with sorghum. Sorghum is also called as great millet; Indian millet belongs to the family Poaceae. It has high antioxidant level compared to other grains and vegetables.

In this study six formulations S1, S2, S3, S4, S5and S6 containing different composition of sorghum flour [0%, 20%, 40%, 60%, 80% and 100%] was developed. These formulations were analysed for sensory attributes.

Further, standardization of fermentation time and analysis of proximate composition was also carried out. Punugulu developed with 40% sorghum and fermented for 12h had highest sensory scores. Punugulu prepared from sorghum had more protein, fibre, calcium, iron and phosphorus and less carbohydrate. They are gluten free and have low glycemic index compared to traditional Punugulu prepared with rice.

Keywords:

Sorghum, Fermentation, Antioxidant, Phytochemicals, gluten free, low glycemic index.

9.1 Introduction:

Sorghum is well-known for its ability to grow in a variety of environments [1]. Sorghum grain is a good choice since it is gluten-free, high in resistant starch, and a good source of minerals, as well as a variety of bioactive phenolic compounds [2].

Bioactive chemicals in sorghum grains enhance the gut microbiota and exhibit a wide range of biological activities [3-5]. In health promotion and disease prevention, sorghum appear to be of major importance for their antioxidant activity [6]. Sorghum induces satiety, reduces calorie intake, and produces a low glycemic response, all of which are beneficial to obese and diabetic individuals [7].

Punugulu is also known as punukulu. It is crispy, crunchy and fluffy snack made by rice, urad dhal and spices. They are often served with peanut chutney. It is common street food of Andhra Pradesh.

9.2 Objectives:

- To develop Punugulu by replacing rice with sorghum and its organoleptic evaluation.
- To analysing its nutritional composition.
- To determine the optimum fermentation time.

9.3 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 sorghum, black gram dhal, cumin seeds, oil and salt were procured from local market of Mysuru.

Method of preparation: Punugulu was prepared by soaking sorghum and Black gram dhal for 4 hours. Rinse the soaked sorghum and dhal and drain the water. Grind the sorghum and dhal, make it into thick batter, leave it for 12 hours.

After fermentation, to the batter add cumin seeds and salt, mix well. On a medium flame, heat oil in a kadai for deep frying, Shape the batter to round balls with fingers and drop them gently in the oil fry the Punugulu until golden and crisp.

Optimisation of fermentation time: Fermentation of Sorghum millet & Black gram dhal of selected variation were allowed to soak for 4 hours. After soaking they were grinding and allowed to ferment for 6, 12, 18, 24 hours respectively at room temperature.

- **Determination of batter volume and pH**: The change in volume & pH, were determined for all the variations at room temperature by varying the time of fermentation. The pH was determined by use of a pH paper and volume was determined by pouring the batter into 250 ml measuring cylinder and recording the volume after 6, 12, 18 and 24 hrs.
- **Sensory Analysis of Prepared Punugulu:** 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 average score of the 20 semi trained panelists by using 9-point hedonic scale.
- **Nutritional analysis of Prepared Punugulu:** Standard A.O.A.C. (1980) method was used to determine the Nutritional composition of selected variation (SP3) of Sorghum Punugulu and control (8). 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 (9,10,11). 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). These methods give a good precision and accuracy (12, 13, 14, 15).
- **Statistical analysis:** Each sample was analysed in triplicates. The data obtained was analysed 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 Formulation of the Product:

Table 9.1: Formulation of the product (ingredients g/100 gm) for preparation of Sorghum Punugulu

Ingredients	**20%**	40%	**60%**	**80%**	**100%**
Sorghum Millets (g)	20	40	40	40	100
Black gram dhal (g)	80	60	60	60	-
Cumin seeds (g)	1.5	1.5	1.5	1.5	1.5
Salt (g)	2	2	2	2	2
Oil (ml)	15	15	15	15	15

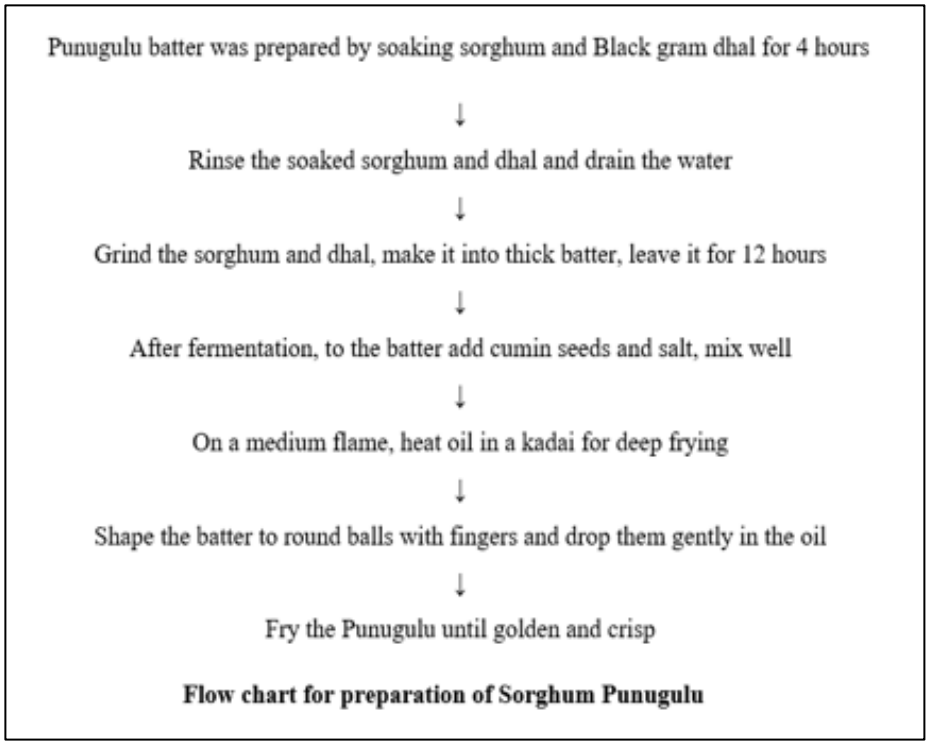

Figure 9.1: Flow chart Preparation of Sorghum Pungulu

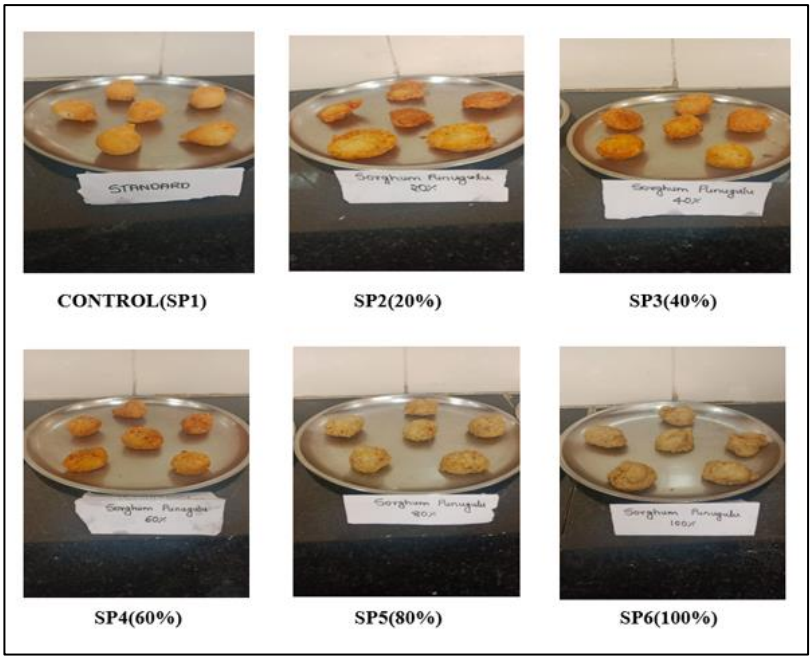

Figure 9.2: Different variations of Punugulu developed from Sorghum in comparison of rice Punugulu.

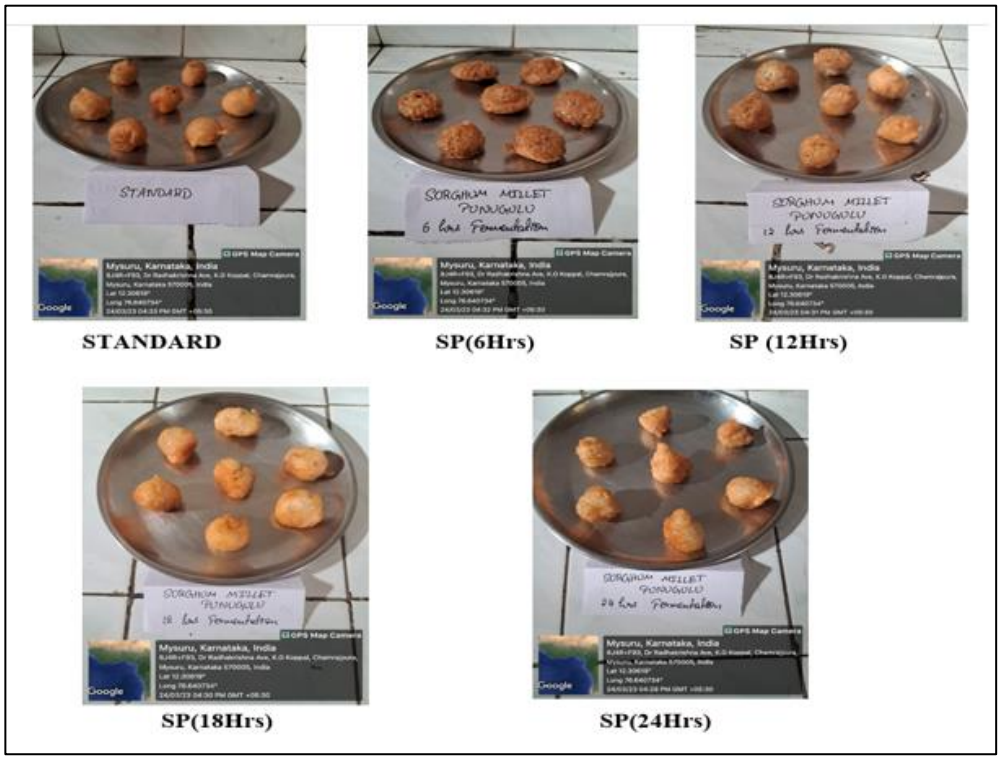

Figure 9.3: Sorghum Punugulu developed by varying the time of fermentation

9.5 Results and Discussion:

The study was undertaken to standardize the fermentation time and quality evaluation of Punugulu prepared by replacing rice with Sorghum and its nutritional analysis.

- **pH and volume of Sorghum Punugulu batter:** The pH value of Punugulu 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 are shown in Table 9.2. There was an increasing in acidity level due the development of Lactic acid bacteria producing lactic acid which lowers the pH, and production of carbon dioxide, which leavens the batter. Initial volume of the batter was 40 ml for all the variations and increased gradually are shown in Table 3. This was due to incorporation of the lactic acid bacteria into the batter during fermentation and entrapment of air.

Table 9.2: pH of Sorghum Punugulu batter.

Variations	Initial pH	pH after 6hrs	pH after 12hrs	pH after 18hrs	pH after 24hrs
Standard (SP1)	6	6	5	5	5
SP2 (20%)	6	6	5	5	5

Variations	Initial pH	pH after 6hrs	pH after 12hrs	pH after 18hrs	pH after 24hrs
SP3 (40%)	6	6	5	5	5
SP4 (60%)	6	6	5	5	5
SP5 (80%)	6	6	5	5	5

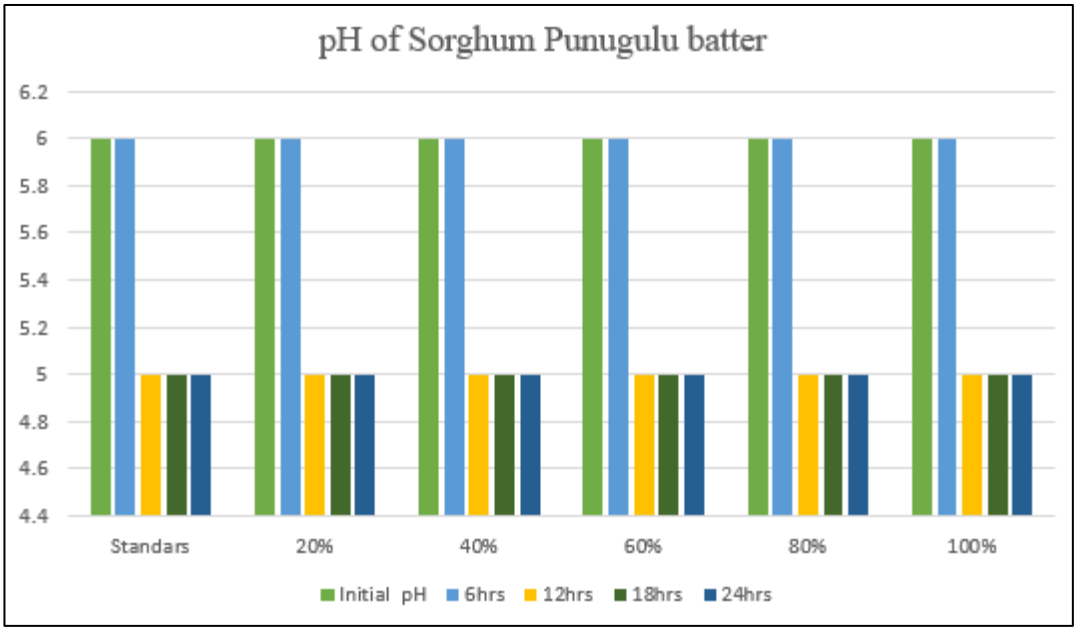

Figure 9.4: Graphical representation of pH of Sorghum Punugulu batter

Table 9.3: Determination of volume of Sorghum Punugulu batter

Variations	Initial Value (ml)	Initial Value after 6 (ml)	Initial Value after 12 (ml)	Initial Value after 18 (ml)	Initial Value after 24 (ml)
SP2 (20%)	40	52	55	56	58
SP3 (40%)	40	55	65	75	78
SP4 (60%)	40	53	57	59	62
SP5 (80%)	40	48	50	55	57
Standard (SP1)	40	50	56	58	60

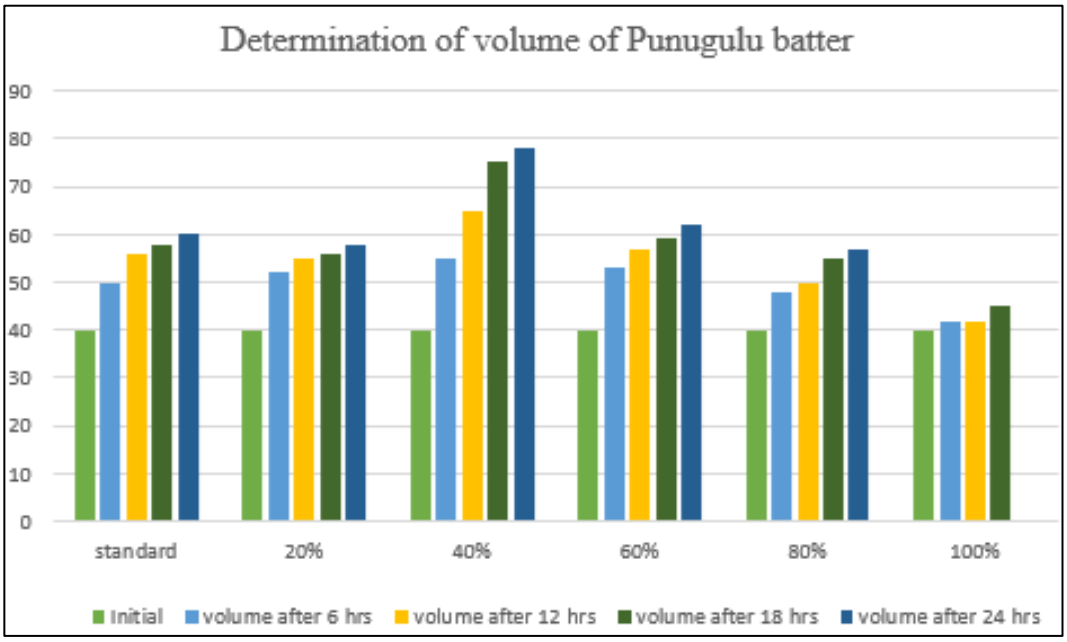

Figure 9.5: Graphical representation of volume of Sorghum Punugulu batter

- **Sensory evaluation of Sorghum Punugulu prepared by varying the proportions:** The data pertaining to the effect of incorporating various levels of sorghum (20%, 40%, 60%, 80%, and 100%) on the sensory attributes of Punugulu are shown in Table 4. The scores obtained for all sensory attributes for SP2, SP3, and SP4 were almost similar and on par with the control. SP5 and SP6 showed decreased scores and were less acceptable compared to the other variations. The variations SP2, SP3, and SP4 had a crisp texture, which was achieved through deep frying in oil. Among all the variations, SP3 had more acceptability in terms of sensory attributes.

Table 9.4: Sensory scores of different variations of Punugulu developed from Sorghum.

Parameters	SP (6h)	SP (12h)	SP (18h)	SP (24h)
Appearance	9.1±0.51	8.5±0.61	8.6±0.53	8.5±0.63
color	8.9±0.41	8.7±0.71*	8.5±0.61	8.3±0.50*
Texture	9.0±0.62	8.5±0.62	8.4±0.32	8.2±0.32*
Taste	9.0±0.71	8.6±0.53	8.5±0.51	8.4±0.51
Flavor	8.9±0.41	8.8±0.33	8.7±0.66	8.5±0.41
Overall acceptability	9.0±0.71	8.6±0.41	8.8±0.71	8.6±0.51

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

- **Sensory evaluation of Punugulu prepared by varying fermentation time:** The standardization of fermentation time for the selected variation resulted in higher acceptability in terms of sensory attributes, with the maximum scores obtained for SP (12h), as shown in Table 5. The optimum fermentation time for sorghum-incorporated Punugulu was found to be 12 hours. Due to inadequate fermentation, SP (6h) had the lowest sensory scores, while the last variation had excessive fermentation, resulting in unfavourable sensory characteristics.

Table 9.5: Sensory scores of Punugulu developed from Sorghum by varying fermentation time.

Parameters	SP (6h)	SP (12h)	SP (18h)	SP (24h)
Appearance	8.2±0.21	8.6±0.33	8.1±0.11	8.2±0.21
Color	8.3±0.31	8.5±0.21	8.2±0.31	8.1±0.31
Texture	8.1±0.21	8.4±0.32	8.3±0.21	8.2±0.21
Taste	8.0±0.12	8.5±0.21	7.9±0.12	7.8±0.12
Flavor	8.1±0.21	8.7±0.36	8.0±0.21	8.0±0.21
Overall acceptability	8.3±0.12	8.8±0.71	8.2±0.12	8.1±0.11

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

- **Proximate Composition of prepared Punugulu:** The proximate composition of the accepted Punugulu (SP3) and the control was analysed, and the results are shown in Table 6. The moisture content of all variations of Punugulu was similar. The protein and fat content in SP3 were higher compared to the control, while the carbohydrate content was lower. However, the fibre, ash, iron, calcium, and phosphorus content were increased in Sorghum Punugulu compared to the traditional rice Punugulu.

Table 9.6: Proximate composition of Punugulu (control and SP3) developed from Sorghum.

Nutrients	Standard Punugulu	SP3(40%)
Moisture (%)	25.03±2.12	28.24±2.81
Carbohydrate (g)	52.11±1.10	40.48±1.60*
Protein (%)	10.48±0.71	15.84±0.83*
Fat (g)	10.30±2.15	11.60±0.28
Crude Fibre (%)	0.59±0.13	1.52±0.30
Ash (g)	1.46±0.11	2.28±0.10
Energy (kcal)	343.06±3.11	329.68±1.81
Iron (mg)	3.507±0.11	4.56±0.32
Phosphorus (mg)	198.16±1.19	224.46±2.02*

Values are mean ± SD (n=3) *p value < 0.05 (Holm Sidak method

9.6 Conclusion:

Punugulu also known as punukulu is a traditional fermented deep-fried snack from Andhra Pradesh. It is crispy, crunchy and fluffy snack made from rice, urad dhal and spices. Rice is rich in carbohydrate and high GI food, Sorghum contains low carbohydrate, gluten free and rich in antioxidants, fibre and minerals. In our study Punugulu prepared with sorghum up to 40% was acceptable. On par with control, the selected Sorghum Punugulu was superior nutritionally and it is high fibre and low carbohydrate makes it a low glycaemic index food, it helps to keep a healthy weight and manage diabetes. Low GI foods could be a tool for healthy life and being gluten free makes it a delicacy to be enjoyed by the people who are gluten intolerant.

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10. Evaluation of Fermentation Conditions to Improve the Sensory Quality of Foxtail Millet (*Setaria Italica*) Idli

Koushik G. C.

PG student,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Manasa R.

Research Scholar,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Shekhara Naik R.

Professor and Head,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Mahesh Shivananjappa

Assistant Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Abstract:

Idli is the most popular fermented breakfast consumed in India. Fermentation is one of the old methods that are used for food preservation. Fermentation evidently decreases the tannin content when millets were incorporated in idli. Foxtail millet (Setaria italica) is one of the oldest crops that consists of high levels of fiber, minerals, protein, phytochemicals, and other bio-active compounds. The present study was conducted to formulate nutritious rich millet idli and comparison of the sensory qualities of different compositions and fermentation times.

In this study, different compositions of idlis (F1, F2, F3, F4, F5, F6) were prepared by incorporating Foxtail millet (0%, 20%, 40%, 60%, 80%, 100%) with Parboiled rice and Black gram dhal. The batter was fermented for 12 hours. Overall acceptability in the sensory evaluation was analysed by semi-trained panellists using the hedonic scale method (n=20). Further, evaluation of fermentation time and an analysis of the proximate composition of the product were carried out. Fermentation of the developed product was evaluated by varying fermentation times (6 h, 12 h, 18 h, 24 h) followed by their sensory evaluation. Idli prepared by incorporating 60% foxtail millet and fermented for 12 h had the highest scores in terms of sensory evaluation. Proximate analysis of acceptable variation had increase in protein, iron, and phosphorous levels and decrease in carbohydrates level compared to standard. This study helps to evaluate the optimum fermentation time and acceptable composition to develop idli by incorporating foxtail millet which possesses hypolipidemic, low glycaemic index, and antioxidant characteristics.

Keywords:

Fermentation, Micro-organisms, Hypolipidemic, Hedonic scale.

10.1 Introduction:

For thousands of years, preserving food after fermentation has been a successful method of increasing the shelf life of food. Traditionally, fermentations that occur naturally were used to preserve food. lactic acid bacteria (LAB) are widely used in a variety of fermented foods, making them a viable option for food improvement. Numerous antagonistic primary and secondary metabolites, such as organic acids, diacetyl, CO2, and even antibiotics, can be produced by these microbes (1). Additionally, members of the group have the capacity to produce a variety of bacteriocins, some of which are active against diseases that affect food, including *Listeria monocytogenes* and *Clostridium botulinum* (2).

Foxtail millet (*Setaria italica*) is one of the most ancient crops to be cultivated, widely grown in the arid and semi-arid parts of Asia and Africa as well as in several other economically developed nations of the world. Significant amounts of protein, fibre, minerals, and phytochemicals can be found in foxtail millet.

By employing the right processing techniques, antinutrients like phytic acid and tannin found in this millet can be reduced to undetectable levels. Additionally, the millet is said to have a low glycaemic index, antioxidant, and hypolipidemic properties (3). It is logical to assume that foxtail millet has a promising future in improving dietary quality and food security (4).

An Indian dish called an idli is made by steaming a batter made of fermented black gram (*Phaseolus mungo*) and rice (*Oryza sativa*). Compared to raw, unfermented ingredients, it contributes significantly to the diet as a source of protein, calories, and vitamins, particularly B-complex vitamins. It can be prepared locally and used as a dietary supplement to treat individuals with protein-calorie malnutrition and kwashiorkor in underdeveloped nations (5).

The naturally existing bacteria in grains, legumes, and utensils proliferate quickly during the overnight fermentation of idli batter, outnumbering the initial contaminants and taking control of the fermentation. These microbes cause the batter to become anaerobic and leaven the final product by producing lactic acid and carbon dioxide (6).

10.2 Objectives:

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

10.3 Materials and Methods:

A. 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, Foxtail millet, Black gram dhal and salt were procured from local grocery shop in Mysuru.

B. Methods:

a. Preparation of Foxtail Millet (FM) Idli: Idli was prepared by replacing parboiled rice with FM in different proportions. Parboiled rice, FM and black gram dhal were soaked for 4 hours. Grind the Parboiled rice and FM into coarsely slurry and black gram dhal into fine paste. Combine both the slurries into thick batter and mix well by adding salt. Allow the batter to ferment for 12 hours in warm place (34-37 °C). Pour the fermented batter into small cups of idli cooker, steam it for 20 minutes.

b. **Optimization of Batter Fermentation:** After addition of salt 2% of total weight of raw material, the batter of selected variation was allowed to ferment for different period (6, 12, 18, 24 h) in aszx stainless-steel vessel. No effort was made to control the temperature during the process of fermentation.

c. pH and Volume: For the different fermentation time of selected variation 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.

d. 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 9-point hedonic scale method.

e. Nutritional Analysis of Idli: Standard AOAC (1980) method was used to determine the nutritional composition of selected variation of FM idli and control. The moisture content was estimated by using hot air oven method (98 to 100 ˚C), Protein content was estimated by determining total nitrogen content using standard Micro-Kjeldahl method, ash % was estimated by high temperature incineration using muffle furnace and fat content was estimated by Soxhlet method. The crude fibre content was estimated by using crude fibre 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 analysed using inductively

coupled plasma mass spectrometry (ICPMS). These methods give good precision and accuracy (7,8).

f. **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).

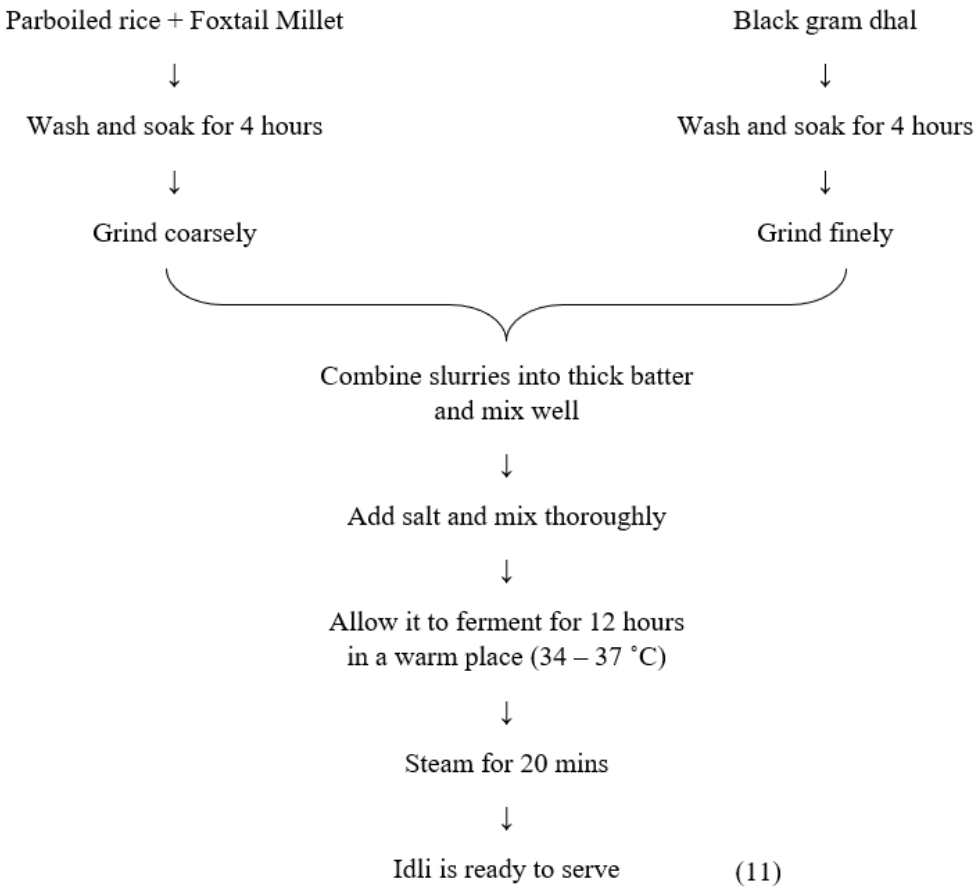

Figure 10.1: Flow chart for preparation of FM idli

10.4 Formulation of the Product:

Table 10.1: Formulation of ingredients (g/100 g) for preparation of FM idli.

Ingredients	F1 (Standard)	F2 (20%)	F3 (40%)	F4 (60%)	F5 (80%)	F6 (100%)
Foxtail 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

10.5 Result and Discussion:

This study helps to evaluate the acceptable composition and fermentation time to develop foxtail millet idli which possess hypolipidemic, low glycaemic index and antioxidant characteristics. The data pertaining to the effect of incorporation of various levels of foxtail millet (0%, 20 %, 40 %, 60 %, 80 % and 100 %) on sensory attributes of idli and the results are shown in Table 10.2. Idli prepared by incorporating 60 % foxtail millet had highest scores in terms of sensory evaluation. The scores obtained for sensory attributes for F4 were almost similar on par with the control.

Table 10.2: Sensory evaluation of different variation of Idli developed from incorporation of FM with Parboiled rice, Values are mean ± SD, n=20 *p < 0.05 (Holm sidak method)

Attributes	F1 (Standard)	F2 (20%)	F3 (40%)	F4 (60%)	F5 (80%)	F6 (100%)
Appearance	8.6±0.5	8.4±0.5	8.1±0.5	**8.3±0.6**	7.6±0.4*	7.5±0.5*
Color	8.6±0.4	8.5±0.5	8.2±0.6	**8.3±0.4**	7.9±0.2*	7.3±0.4*
Texture	8.6±0.4	8.5±0.5	8.0±0.4	**8.2±0.8**	7.6±0.4*	7.2±0.4*
Taste	8.2±0.7	8.5±0.6	8.1±0.6	**8.4±0.6**	7.7±0.4	7.4±0.5
Flavor	8.2±0.6	8.3±0.6	8.2±0.6	**8.2±0.5**	7.6±0.4	7.2±0.4
Overall acceptability	8.2±0.5	8.4±0.5	8.1±0.6	**8.4±0.5**	7.9±0.2	7.3±0.4

F1(Standard) **F2** **F3**

F4 **F5** **F6**

Figure 10.1: Different Variations of Idli Developed from FM In Comparison to Parboiled Rice Idli

Further, the acceptable composition was prepared by varying their fermentation time. The data pertaining to the effect of different fermentation time on foxtail millet idli and the results are shown in Table 10.3.

Fermentation of the developed product was evaluated by varying the fermentation time (6 h, 12 h, 18 h, 24 h) followed by their sensory evaluation. The results showed that the optimum fermentation time for Foxtail millet incorporated idli was found to be 12 h.

Table 10.3: Sensory evaluation of FM idli incubated for different fermentation time, Values are mean ± SD, n=20 *p < 0.05 (Holm Sidak Method).

Attributes	Standard	6 h	12 h	18 h	24 h
Appearance	8.6±0.5	7.0±0.7*	**7.6±0.8**	7.5±0.5*	7.0±0.7*
Color	8.6±0.4	7.3±0.7*	**7.8±0.7**	7.8±0.5	7.1±0.6*
Texture	8.6±0.4	7.1±0.5*	**7.8±0.5**	7.5±0.6*	6.8±0.7*
Taste	8.2±0.7	6.9±0.4*	**7.8±0.5**	7.7±0.4	6.8±0.6*
Flavor	8.2±0.6	6.9±0.4*	**7.7±0.7**	7.8±0.5	6.6±0.6*
Overall acceptability	8.2±0.5	6.9±0.4*	**7.9±0.5**	7.8±0.5	6.7±0.4*

6 hr

12 hr

18 hr

24 hr

Figure 10.2: FM incorporated idli prepared by varying fermentation times.

10.6 pH Value:

The pH of the FM idli batter was measured using the pH paper. The fermented FM idli batter of selected variation (12 h) showed the pH of 5, which shows the acidic nature. Acidic Ph in idli is primarily due to the fermentation process. Lactic acid fermentation is mainly responsible for the sour taste and acidic pH of idli 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 idli 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 if idli.

Table 10.4: pH of FM incorporated (F4) idli batter fermented for different times.

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

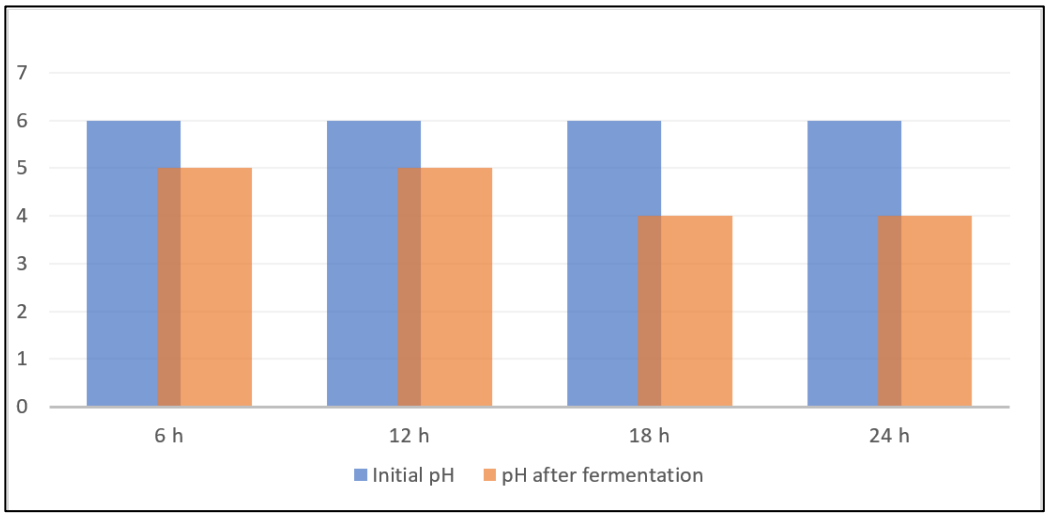

Figure 10.3: Determination of pH of FM incorporated (F4) idli batter.

10.7 Volume:

The difference in volume was measured in the FM idli batter before and after optimum fermentation in the batter. The batter was fermented in a warm place (30 to 32^{0C}) for 6, 12, 18, 24 hours. The volume of the batter of selected variation (12 h) before fermentation was 60 ml and the volume of the batter after fermentation was 78 ml. 18 ml rise in the batter was observed after fermentation. 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 idli.

Table 10.5: Volume of FM incorporated (F4) idli batter fermented for different times.

Variations	Initial volume (ml)	Volume after fermentation (ml)
6 h	60	73
12 h	60	78

Variations	Initial volume (ml)	Volume after fermentation (ml)
18 h	60	84
24 h	60	90

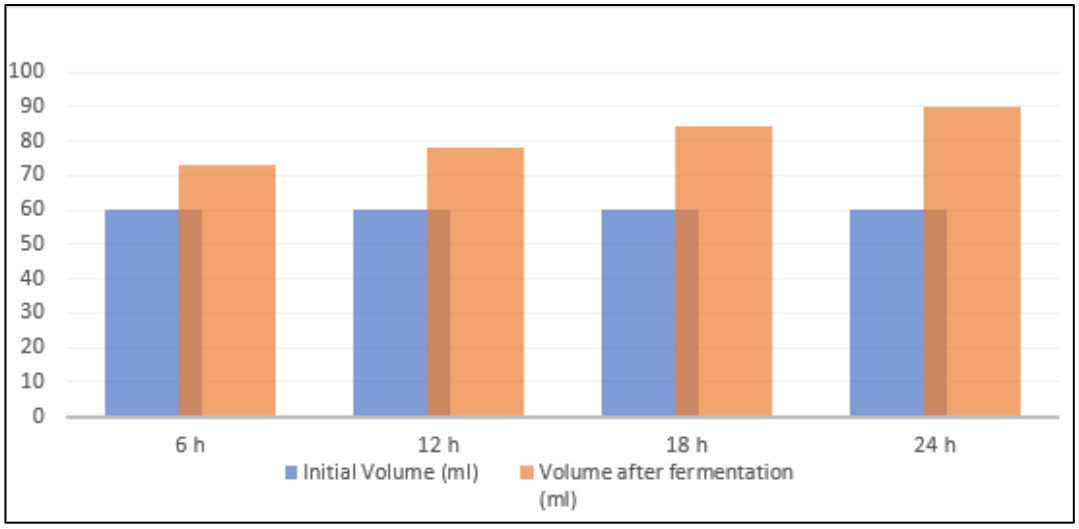

Figure 10.4: Determination of volume of FM incorporated (F4) idli batter.

10.8 Proximate Analysis

Proximate analysis of the acceptable variation of FM idli, F4 (60 %, 12 h) and that of control were analysed. The results are shown in Table 6. Acceptable variation had increase in protein, iron and phosphorous levels compared to standard, whereas carbohydrates level was decreased.

Table 6: Proximate analysis of selected variation (F4) of FM incorporated idli.

Nutrients/ 100 g	Standard	F4 (60%)
Moisture (%)	23.34±0.15	20.3±1.58
Ash (g)	1.54±0.02	1.92±0.06
Energy (kcal)	301.16±0.18	321.28±0.19*
Carbohydrates (g)	61.68±0.85	59.2±0.45
Protein (g)	11.81±1.10	16.17±2.09*
Fat (g)	0.8±0.35	2.2±0.28*
Crude Fiber (g)	0.83±0.03	0.21±0.05
Phosphorous (mg)	95.3±0.12	174±0.8*
Iron (mg)	1.86±0.14	3.5±0.04*

Values are mean ± SD, n=3 *p < 0.05 (Holm Sidak Method).

10.9 Conclusion:

Foxtail millet idli was developed in this study by varying formulation and fermentation times. Idli was chosen for this product development, because of the wide acceptance of idli among the consumers. Organoleptic evaluation of idli revealed that FM (60 %) with 12 h fermentation time had high acceptance for its appearance, colour, texture, flavour, and taste among all variations. FM (60 %) with 24 h fermentation time had lowest sensory scores due to hyper-fermentation. Fermentation helps in increased digestibility, nutritional value and decreases the tannin content when millets were incorporated in idli. 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, dietary fiber, vitamins, and proteins. So, new value-added products can be developed from millets. The optimum fermentation time for Foxtail millet incorporated idli was found to be 12 h and acceptable up to 60 % which possess hypolipidemic, low glycaemic index and antioxidant characteristics. Proximate analysis of acceptable variation had increase in protein, iron and phosphorous levels and decrease in carbohydrates level compared to standard.

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11. Effect of Fermentation on Sensory Parameters of Sorghum Incorporated Dhokla (*Sorghum Bicolour*)

Kusum N.

PG student,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Manasa R.

Research Scholar,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Shekhara Naik R.

Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Mahesh Shivananjappa

Assistant Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Abstract:

Fermentation technique is traditionally practiced for the preparation of food with the help of microorganisms and their enzymes. Fermentation of grains leads to a general improvement in shelf life, texture, taste, aroma, nutritional value, digestibility, and it also reduces anti-nutritional factors while enhancing the bioavailability of nutrients. Sorghum millet is one of the main staple foods, being a rich source of fibre, phenolic compounds, flavonoids, tannins, carotenoids, vitamin E, policosanols, and phytosterols. Dhokla is a Gujarati cuisine made from fermented rice and lentil batter, which is then cooked and

tempered. This study was conducted to develop and evaluate six different variations of fermented Dhokla. These developed products were analysed for sensory attributes, with the sensory score being highest for SMD3 (40%), making it highly acceptable. Furthermore, the fermentation time of the selected variation was varied, and the 12-hour fermented dhokla was highly acceptable. Subsequently, they were subjected to proximate analysis, resulting in increased carbohydrate, fat, crude fibre, ash, energy and iron content in SMD3, on par with the control.

Keywords:

sorghum bicolour, jowar, lactic acid bacteria, Dhokla

11.1 Introduction:

Fermented foods are defined as "foods or beverages manufactured by controlling microbial growth and converting food constituents through enzymatic activity, converting sugar to alcohol and acid anaerobically, liberating energy" (1). Dhokla is a traditional legume-cereal based fermented food, popular in Gujarati cuisine due to its texture. There are different types of dhokla, such as Khaman Dhokla and Katta Dhokla. The leavening of the batter is a result of acid development, indicating fermentation. Lactic acid bacteria (LAB) play a crucial role in developing the characteristic flavour of dhokla, while yeasts contribute to increasing the batter volume by producing folic acid, resulting in the spongy texture when cooked in steam (3). There are two main types of fermentation: lactic acid fermentation and alcohol fermentation (1). Dhokla is developed by adding a starter culture known as "culture-dependent ferments" (2). Fermentation improves the nutritional quality by reducing anti-nutritional factors and enhancing the bioavailability and digestibility of nutrients. It also improves the texture, taste, shelf life, and aroma of the food (3). Lactate also modifies oxidative stress levels by reducing accumulated reactive oxygen species in intestinal enterocytes (11). Sorghum is one of the nutri-cereals that are generally rich in phytochemicals, particularly various types of phenolic compounds. Different food processing operations like dehulling, decortication, malting, thermal processing, and fermentation help reduce the effects of phenolic compounds and phytates present in sorghum. These compounds contribute to maintaining blood glucose levels and preventing cardiovascular disease and cancer through their phytochemical action (4) (5).

11.2 Objectives:

- To develop sorghum-based dhokla by partially replacing the Bengal gram dal with sorghum.
- To evaluate the organoleptic parameters of the developed product.
- To determine the optimum fermentation time for the developed product.

11.3 Materials And Methods:

A. Raw materials: The present study was conducted in the Department of Food Science and Nutrition at Yuvraja's College, an autonomous institution affiliated with the

University of Mysore, located in Mysuru. The raw materials used in the study were procured from the local market of Mysuru and included sorghum millet, Bengal gram dal, curd, salt, oil, turmeric powder, chilies, mustard seeds, curry leaves, and sugar.

B. Method of preparation: The standardization of Dhokla was conducted by varying the proportion of sorghum millet and Bengal gram dal. The cleaned sorghum millet and Bengal gram dal were soaked in water for 6 hours, and then ground into a fine paste. Water was added to achieve the desired consistency. Salt (NaCl) and curd were added to the mixture, and it was allowed to ferment for 12 hours in an anaerobic condition at room temperature. To the fermented batter, a pinch of turmeric powder, 1 teaspoon of green chili paste, and a pinch of soda were added. The mixture was then steam cooked for 30 minutes and cut into square pieces. For seasoning, oil, mustard seeds, asafoetida, curry leaves, and green chilies were used. Additionally, a mixture of sugar and water was prepared and poured over the dhokla to dissolve the sugar.

C. Optimisation of fermentation time: The fermentation of the selected variation of Sorghum millet and Bengal gram dal involved soaking them for 6 hours. After the soaking process, they were allowed to ferment for different durations of 6, 12, 18, and 24 hours, respectively, at room temperature [10].

D. Determination of volume and pH: The change in volume and pH of all the compositions was determined at room temperature by varying the fermentation time. The pH was measured using pH paper, and the volume was measured by pouring the batter into a 250 ml measuring cylinder and recording the increase in volume after 6, 12, 18, and 24 hours of fermentation.

E. Sensory analysis of Dhokla: The developed dhokla was evaluated for its organoleptic properties, including appearance, colour, texture, taste, flavour, and overall acceptability. A sensory evaluation was conducted with the participation of 30 semi-trained panellists. A 1 to 9 hedonic scale was used for rating the quality of the dhokla, with higher scores indicating greater satisfaction. The mean value of the 30 scores was considered for evaluation, providing an average assessment of the sensory attributes of the dhokla.

F. Nutritional analysis of prepared Dhokla: The proximate estimation of the chosen variation and control dhokla was carried out following the standard AOAC (1990) methods, which are known for their accuracy and precision. The moisture content was evaluated using a hot air oven set at 98-100°C. The protein content was estimated using the Micro-Kjeldahl method, which determines the total nitrogen content.

G. Fat content was analysed using the Soxhlet method. The ash content was estimated by incinerating the food sample at a high temperature (550°C for 6 hours) in a muffle furnace. Crude fibre content was determined using a Crude Fibre Analyser. The carbohydrate content was calculated by subtracting the sum of moisture, protein, fat, and ash content from 100 grams of the sample (6). Phosphorus and iron content were estimated using Inductively Coupled Plasma Mass Spectrometry (ICPMS) (7).

H. Statistical analysis: Each sample was analysed in triplicates to ensure reliable and consistent results. The obtained data was subjected to statistical analysis using the standard methods provided by Snedecor and Cochran (8). To determine significant differences between the samples, Duncan's multiple range test was applied, with a significance level of $p \leq 0.05$ considered to be statistically significant (8) (9). This analysis allowed for proper comparison and interpretation of the data, identifying any significant variations among the samples.

11.4 Formulation of the Product:

Table 11.1: formulation of the products (ingredients g/100g) for preparation of sorghum millet dhokla.

Ingredients	Standard	20%	40%	60%	80%	100%
Sorghum millet (g)	-	20	40	60	80	100
Bengal gram dal (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
Sugar (g)	15	15	15	15	15	15
Chillies (g)	2	2	2	2	2	2

Sorghum millet and Bengal gram dhal are soaked in water for 6 hours.

↓

After soaking, the sorghum millet and Bengal gram dhal are ground into a fine paste. Water is added as needed to adjust the consistency.

↓

Salt (NaCl) and curd (yogurt) are added to the ground paste. The mixture is allowed to ferment for 12 hours in an anaerobic condition at room temperature. Fermentation helps in making the dhokla soft and adds flavour.

↓

To the fermented batter, a pinch of turmeric powder, 1 teaspoon of green chili paste, and a pinch of soda (sodium bicarbonate) are added. These ingredients enhance the taste and texture of the dhokla.

↓

The batter is then steamed for approximately 30 minutes until it becomes cooked and firm. After steaming, the dhokla is cut into square pieces.

↓

In a separate pan, oil is heated, and mustard seeds, asafoetida (hing), curry leaves, and green chilies are added. A mixture of sugar and water is also added to dissolve the sugar. This seasoned mixture is then poured on top of the steamed dhokla.

Figure 11.1: Flow chart for preparation of sorghum millet based dhokla.

Figure 11.2: Dhokla Prepared by Varying the Composition of Sorghum & Bengal Gram Dhal

Figure 11.3: Dhokla Prepared by Varying the Time of Fermentation.

11.5 Results & Discussion:

The study focused on standardizing the fermentation time for Dhokla and evaluating its sensory parameters by partially replacing Bengal gram dhal with Sorghum millet. Here are the key findings and observations from the study:

pH Value: The pH of the Dhokla batter ranged from 5.0 to 6.0 during different fermentation periods (6, 12, 18, and 24 hours). The initial pH was 6.0, and as fermentation occurred, the pH consistently decreased to 5.0. This decrease in pH was due to the development of lactic acid bacteria, which produces lactic acid, thereby lowering the pH. The production of carbon dioxide during fermentation also contributed to leavening the batter. Volume Changes: The initial volume of the batter was 40 ml for all variations and gradually increased until 18 hours of fermentation. However, at 24 hours, the volume of the batter decreased. The increased volume during fermentation can be attributed to the growth of lactic acid bacteria and the entrapment of air. Sensory Evaluation with Sorghum Millet: Different levels of sorghum millet (20, 40, 60, 80, and 100%) were incorporated into the Dhokla batter. The sensory attributes were evaluated, and it was found that scores for sensory attributes in SMD2, SMD3, and SMD4 were similar to the control (BGD1). SMD5

and SMD6 received lower scores and were less acceptable due to their hard texture. SMD3 was highly acceptable in terms of all sensory attributes. Sensory Evaluation with Fermentation Time: Dhokla variations with SMD3 were fermented for different durations (6, 12, 18, and 24 hours). It was observed that Dhokla fermented for 12 hours received the highest acceptance scores for sensory attributes. Dhokla with 6 hours of fermentation had poor texture, taste, and colour due to insufficient fermentation. Over-fermentation occurred in Dhokla with 18 and 24 hours of fermentation, resulting in sourness, collapse in structure, and reduced sensory scores.

Proximate Analysis: The highly acceptable Sorghum Millet Dhokla (SMD3) and the control were subjected to proximate analysis. The results showed that SMD3 had high carbohydrate content and higher fat, crude fibre, energy, ash and iron content compared to the control.

11.5.1 pH of Dhokla Batter:

Table 11.2: pH if dhokla batter.

Variations	Initial pH	pH after 6hrs	pH after 12hrs	pH after 18hrs	pH after 24hrs
Standard	6	5	5	5	5
SMD 20%	6	5	5	5	5
SMD 40%	6	5	5	5	5
SMD 60%	6	5	5	5	5
SMD 80%	6	5	5	5	5
SMD 100%	6	5	5	5	5

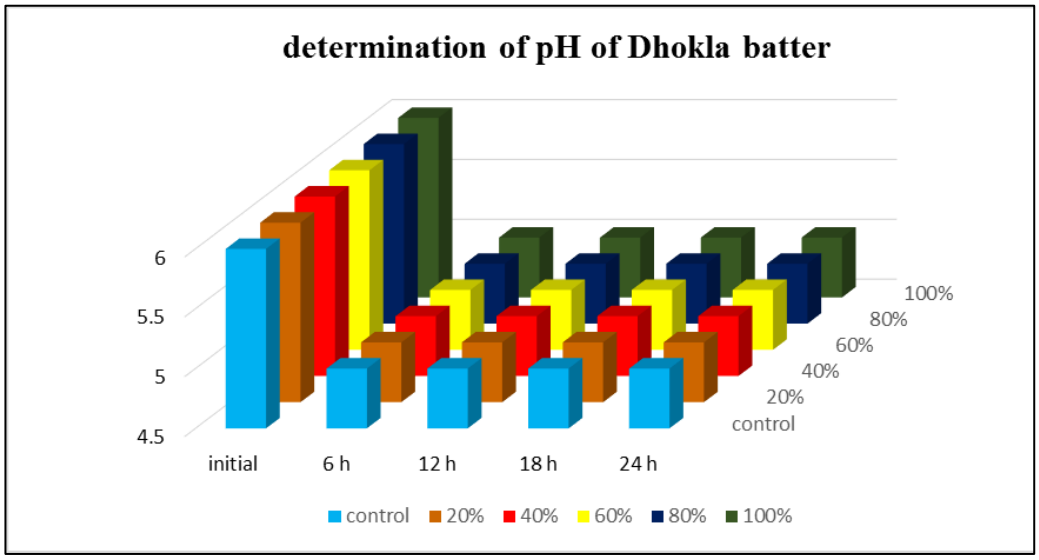

Figure 11.4: pH of Dhokla Batter

11.5.2 Determination of Volume:

Table 11.3: Determination of Volume of Dhokla Batter

Variations	Initial Volume (ml)	Volume after 6hrs	Volume after 12hrs	Volume after 18hrs	Volume after 24hrs
Standard	40	50	55	59	59
SMD 20%	40	50	54	56	56
SMD 40%	40	56	65	78	78
SMD 60%	40	49	50	56	56
SMD 80%	40	45	51	54	54
SMD 100%	40	40	40	40	40

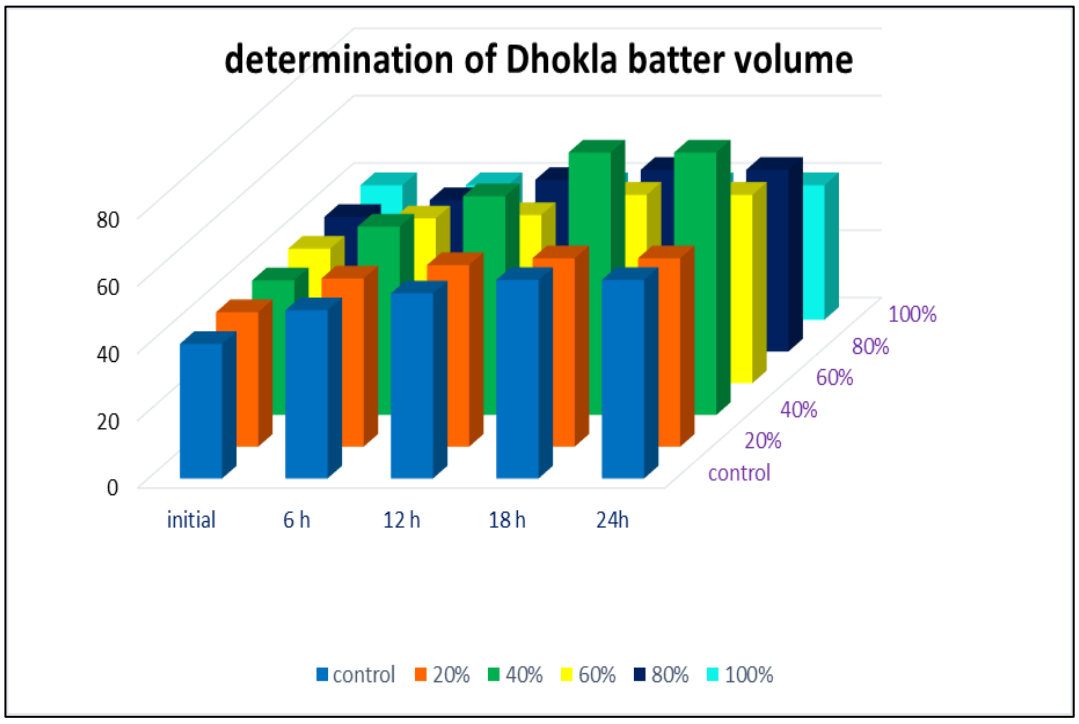

Figure 11.5: Volume of Dhokla Batter

11.5.3 Sensory Scores of Dhokla Prepared by Varying the Proportions:

Values are mean ± SD (n=30) *p value < 0.05 (Holm sidak method).

Table 11.4: sensory scores of dhokla prepared by varying the proportion of Sorghum millet & Bengal gram dhal

Parameters	Standard BGD1	SMD2 (20%)	SMD3 (40%)	SMD4 (60%)	SMD5 (80%)	SMD6 (100%)
Appearance	8.81±0.39	8.30±0.29	8.54±0.50*	8.27±0.45	8.0±0.0	7.5±0.51
Color	8.72±0.45	8.06±0.35	8.36±0.49*	8.22±0.42	7.72±0.45	7.45±0.50
Texture	8.72±0.45	8.10±0.39	8.36±0.58*	8.13±0.46	7.09±0.29	7.31±0.56
Taste	8.68±0.56	8.21±0.59	8.27±0.63*	7.95±0.37	7.36±0.49	6.95±0.84
Flavor	8.68±0.56	8.19±0.55	8.27±0.63*	8.04±0.37	7.31±0.47	7.18±0.66
Overall acceptability	8.72±0.45	8.30±0.42	8.36±0.58*	8.09±0.42	7.36±0.49	7.27±0.55

11.5.4 Sensory scores of Dhokla Prepared by Varying Fermentation Time:

Table 11.5: Sensory Scores of Dhokla Prepared by Varying Fermentation Time

Parameters	SMD06 (6hr)	SMD12 (12hr)	SMD18 (18hr)	SMD24 (24hr)
Appearance	7.81±0.36	8.6±0.51*	7.9±0.96	7.5±0.73
Color	8.01±0.15	8.3±0.52*	8.0±0.48	8.1±0.42
Texture	7.32±0.85	8.6±0.45*	7.8±0.42	7.2±0.24
Taste	7.10±0.23	8.7±0.40*	8.1±0.87	7.1±0.24
Flavor	7.01±0.62	8.5±0.52*	7.9±0.51	7.0±0.46
overall acceptability	7.2±.54	8.8±0.42*	8.0±0.42	7.0±.72

Values are mean ± SD (n=30) *p value < 0.05 (Holm sidak method).

11.5.5 Proximate Composition table of Dhokla (SMD3):

Table 11.6: Proximate Composition Table

Nutrients	Standard	SMD3
Moisture (%)	21.07±0.36	20.96±0.56
carbohydrate (g)	57.88±0.15	58.76±0.41*
protein (g)	12.18±0.65	10.36±0.23
fat (g)	5.82±0.32	6.24±0.14*
Crude fibre (g)	0.55±0.10	0.85±0.10*
ash (g)	2.50±0.02	2.83±0.22*
energy (kcal)	332.62±0.42	332.64±0.51*
iron (mg)	4.43±0.13	5.35±0.24*
Phosphorus (mg)	302.71±0.28	301.3±0.32

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

11.6 Conclusion:

Dhokla is a popular Indian snack originating from Gujarat. In this study, the researchers aimed to develop dhokla using sorghum as the main ingredient. The fermentation process was employed to not only enhance the nutritional value of the product but also prevent food spoilage. Fermentation has the added advantage of reducing anti-nutritional factors such as phytates, tannins, and polyphenols. Additionally, it increases the bioavailability of nutrients and can even enhance the vitamin C content. The nutritional composition analysis of the sorghum dhokla revealed that it is rich in crude fibre. This high fibre content is beneficial for preventing constipation and can also help in reducing LDL cholesterol levels by binding to it and eliminating it from the body through faeces. Thus, sorghum dhokla can be a healthy snack option. The study also found that the dhokla prepared with 40% sorghum content was highly acceptable. This suggests that incorporating sorghum as a partial replacement for other ingredients in dhokla recipes can yield a nutritious and well-liked final product.

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12. Anti-Bacterial Activity of Aloe Vera (*Aloe Barbadensis*)

Maheshwari K. M.

PG student,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Manasa C.

PG student,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Manasa R.

Research Scholar,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Shekhara Naik R.

Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

Mahesh Shivananjappa

Assistant Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysuru, Karnataka, India.

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Abstract:

Aloe barbadensis (Ab) is commonly known as Aloe Vera. It belongs to the family Asphodelaceae. It has medicinal property and been traditionally used for wound healing. Ab has many pharmacological activities such as anti-inflammation, anti-cancer, anti-hyperglycaemic, ant-oxidant and other activity. The present study aims to investigate the inhibitory effect of aloe vera extract against the bacterial growth. The extract of aloe vera leaves was used with different solvents like Ethanol, methanol, Ethyl alcohol extract. The antibacterial activity of the extracts was evaluated by using agar well diffusion method against both Gram-positive and Gram-negative bacteria. The aloe vera extract exhibited inhibitory activity against bacterial strains tested. The zone of inhibition observed ranged from 00 mm per 25 mg/ml to 33.2 mm per 0.50 mg/ml, and the maximum inhibition being observed against E. bovis (33.2 mm). Minimum inhibition showed on P. aeruginosa, T. mentagrophytes, M. Canin. The minimum inhibitory concentration (MIC) of the extract was found to be in the range of 0.10 mg/ml to 100 mg/ml. The above result confirms the inhibitory effect of Aloe Vera extracts and Aloe Vera against several microbes.

Keywords:

Gram positive, leaves extract, Antioxidant, Zone of inhibition

12.1 Introduction:

Aloe Vera has been known for its medicinal properties for centuries [1]. One of its most well-known benefits is its antimicrobial activity, which means that can kill or inhibit the growth of microorganisms such as bacteria, viruses, and fungi. The gel extracted from the inner part of the leaves of aloe vera, and it contains compounds such as anthraquinones, lectins, and polysaccharides, which been shown to have antimicrobial properties. Aloe Vera is also well known for natural dietary supplement and chemo preventive agent. In addition to its antimicrobial activity, aloe vera has other beneficial properties such as anti-inflammatory, wound-healing, and moisturizing effects, making it a popular ingredient in skincare and personal care products [2].

12.2 Morphology of Aloe Vera:

Aloe Vera plant that has a distinctive morphology with several characteristic features. Here are some of the notable features of Aloe Vera's morphology:

Table 12.1: Morphology of Aloe Vera

Leaves	Aloe Vera has long, narrow, succulent leaves that grow in a rosette pattern. The leaves are green and can reach up to 60-90 cm in length and 5-8 cm in width. They are thick and fleshy and have serrated edges with small white teeth [3].

Stem	Aloe Vera has a short, stubby stem that grows from the centre of the rosette of leaves. The stem is usually hidden by the leaves and is not very visible.
Flower	Aloe Vera produces tall, spike-like clusters of tubular flowers that grow from a single stem that emerges from the centre the plant. The flowers are usually yellow, orange, or red and can be up to 90 cm in height. The flowers bloom in the summer and are pollinated
Root	Aloe Vera has a shallow root system that is not very extensive. The roots are fleshy and are used to store water [4].

12.3 Nutritional Composition of Ab:

Aloe Vera has 290.08 kcal for 100g. CHO 56.27 g, Protein and ash 10.50 g and 19.50 g, in whole leaf Calcium is about 3.58 g, Magnesium is 1.22 g respectively [5].15 kcal energy and it also contains, 3.75 g sugar, 8 mg calcium, 0.15 mg iron, 8 mg sodium, 3.8 mg vitamin C [13-14].

Table 12.2: Nutritional Composition of Aloe Vera

Nutritional values of Aloe Vera	Values
Total solids	1.50 %
Moisture	98.50 %
Ash	19.50 g
pH	4.16
Total Protein	10.50 g
Carbohydrates	56.27 g
Sugar	3.75 g
Calcium	8 mg
Iron	0.15 mg
Sodium	8 mg
Vitamin C	3.8 mg
Fat	1.0168 g
Fiber	0.12 g

12.4 Phytochemicals of Ab:

Aloe Vera contains various types of phytochemicals include Polysaccharides, Flavonoids, Sterols, Enzymes, Salicylic acid [6].

12.5 Pharmacological Activities:

Aloe Vera was traditionally used for many medicinal properties. The aloe vera contains various bioactive component are responsible to its pharmacological activities. Some of the pharmacological activity of aloe vera are like reduce inflammation in gums, control nervous

system, reducing wrinkles, curing acne [15], Dermatological, Wound healing, Anti-tumour, Anti diabetic, Anti-inflammatory, etc. [12].

12.6 Antimicrobial Activity of Ab in Bacteria:

Danish *etal.,* (2020) studied antimicrobial activity of both gram positive and gram negative bacteria like *E. coli, A. Baumanni, P.Aerugenosa, S. Typhii, A. Tumefacins, S.Cereus, B.Subitis, B.megaterium, S.Aureus, S.Pygenes* respectively here the bacteria is extracted by the treatment of ethanol extract (10,20,30 µl) and shows the MIC in gram postive P. Aerugenosa about 12mm and in gram negative bacteria *S. Cereus and S. Pygenes* with 13mm zone of inhibition [1]. Dr.V.Anuetal., in the year 2018 studied antimicrobial activity of *S.Aureus , C.Albicans* and here *S. Aureus* is treated with Ethanol extract and shows the zone of inhibition of 9mm and *C. Albicans* used Ethyl alcohol extract (70%). As a result, 12 mm zone of inhibition [11].

Abdul Haq *etal.*, in 2020 studied antimicrobial activity of Ab of both positive and negative bacteria with the extract of ethanol and Methanol extract (35µg/ml) in *E. coli, P.Vulgaris* with the zone of inhibition 16 mm and 14 mm respectively [10].

Ruchi Pandey *etal.,* in 2009 studied antimicrobial activity Ab of Gram-positive bacteria with the extract of ethanol 0.50 mg/ml and the MIC is 20.6 mm and in Gram negative bacteria shows different zone of inhibition with different treatment and the minimum inhibition results in *K. Pneumonae* with 8 mm in the treatment of 10.0 mg/ml [9].

Darshan Dharajiya *etal., (*2017) studied antimicrobial activity, in gram positive bacteria shows MIC in *E.Coli, P.Aeruginosa* 25 mm in 100 mg/ml and in gram negative bacteria the MIC is 12.3 mm with the treatment 100 mg/ml of DMSO [8]. MT Olaleye *etal.,* (2005) also studied antimicrobial activity of bacteria T.Mentagrophytes zone of inhibition is 20 mm, but *P.Aeruginosa, T.Schioeleini and M.Canins, C.Albicans* with the treatment of ethanol extract 25 mg/ml which shows zero zone of inhibition [7].

Table 12.3: Antimicrobial Activity of Aloe Vera [Ab: Aloe Barbadaensis, EEXT: Ethanol extract, MEXT: Methanol extract, Ethyl alcohol, DMSO: Dimethyl sulphoxide]

Sr. No.	Model	Treatment	Result Zone of inhibition	References
01	Gram negative bacteria *E. coli* *A. Baumanni* *P. Aerugenosa* *S. Typhii* *A. Tumefacins* Gram positive *bacteria* *S. Cereus* *B. Subitis* *B. Megaterium*	Ab [EEXT] (10,20,30 µl)	 18 mm 13 mm 12 mm 12.5 mm 18 mm 13 mm 15 mm 14.5 mm	[1]

Sr. No.	Model	Treatment	Result Zone of inhibition	References
	S. Aureus *S. Pygenes*		14 mm 13 mm	
02	*S. Aureus* *C. Albicans*	Ab, Gel [EEXT] Ethylalcohol (70%)	9 mm 12 mm	[11]
03	Gram negative bacteria *E. coli* *P. Vulgaris* *A. Baumanni* *P. Aerugenosa* Gram positive bacteria *S. Aureus* *B. Cereus* *E. Faecalis*	Ab Root [EEXT] [MEXT] 35 µg/ml 35 µg/ml	 16 mm 14 mm 12.5 mm 15 mm 16 mm 14.5 mm 12 mm	[10]
04	Gram positive bacteria *E. Bovis* *S. Aureus* Gram negative bacteria *E. coli* *P. Vulgaris* *P. Aeruginosa* *K. Pneumonae* *M. Morganni*	Ab [EEXT] 0.50 mg/ml 10 mg/ml 0.50 mg/ml 0.10 mg/ml 10.0 mg/ml 0.30 mg/ml	 33.2 mm 20.6 mm 9.6 mm 17.6 mm 19.3 mm 8 mm 24 mm	[9]
05	Gram negative bacteria *E.Coli* *P. Aeruginosa* *S. Marcescens* Gram positive bacteria *B. Cereus*	Ab [MEXT] 100 mg/ml DMSO 100 mg/ml	 25 mm 25 mm 13.6 mm 12.3 mm	[8]
06	*S. Aureus* *P. Aeruginosa* *T. Mentagrophytes* *T. Schioeleini* *M. Canins* *C. Albicans*	Ab [EEXT] 25 mg/ml	18.00 mm 0.0 20.0 mm 0.0 0.0 0.0	[7]

12.7 Conclusion:

The present study has imparted that the ethanol leaf and root extracts of Aloe Vera gel has intended effect of antibacterial activity against both Gram-positive and as well as Gram-negative bacteria. This investigation further assures that the plant extracts could be used for the treatment of microbial infections. It can potentially be used in the development of new antimicrobial agents for the prevention and treatment of various infection diseases.

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11. - Dr.V.Anu MDS*1, Dr. D. Pavani2, E. Sindhuja3, K. Soundarya3, J.M. Shandhinidevi3 1 Reader and Head, Department of Public Health Dentistry, Sathyabama Dental College and Hospital, Chennai, TN, India. 2 BDS, Lecturer, Department of Conservative and Endodontics.
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13. Effect of Fermentation Time on Sensory Attributes of Finger Millet (*Eleusine Coracana L.*)

Manasa C.

PG student,
Dept. of Food science and Nutrition,
Yuvaraja's college, (Autonomous),
University of Mysore,
Mysore.

Manasa R.

Research Scholar,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysore.

Shekhara Naik R.

Professor and Head,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysore.

Mahesh Shivananjappa

Assistant Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysore.

Abstract:

Fermentation is the traditional food processing Technique which increases the bioaccesibility of nutrients. Selroti is a fermented rice-flour confectionary indigenous to Nepal, which is doughnut-like deep fried, puffed, spongy, and ring-shaped. It has good number of digestible proteins. Finger millet (Eleusine coracana L.), provides highest level of calcium which helps in strengthening bones for growing children and aging people, antioxidants properties, phytochemicals, which makes it easily and slowly digestible. This study was conducted to develop and evaluate Selroti of six different compositions F1, F2, F3, F4, F5 and F6 containing jaggery, refined wheat flour, spices with different composition of Finger millet and rice flour (0%, 20%, 40%, 60%, 80%, 100%) were developed and

evaluated for acceptability using subjective and objective method by taking rice flour Selroti as a control. The developed product was analyzed for sensory attributes (n=30). The sensory score was highest in F4. Fermentation of the developed product was optimised and detected by performing variations in fermentation time [3 Hrs, 6 Hrs, 9 Hrs & 12 Hrs], followed by their sensory evaluation. The sensory score was highest for the product which was fermented for 6 Hrs and its nutrition composition was calculated according to standard AOAC method. The present study documents how the microbial growth influence the batter characteristics and quality of Selroti in terms of nutritional and sensory attributes.

Keywords:

Finger millet, fermentation, antioxidant, *Leuconostoc*, phytochemical.

13.1 Introduction:

Fermentation is one of the oldest microbiological techniques for preserving food, improving nutritional value, and improving sensory qualities while retaining enzymes, vitamins, and other nutrients. It aids in the maintenance of a healthy configuration of celiac microbiota. Plant proteins become more digestible after fermentation. Selroti is a tasty energy-dense snack with a medium shelf life (1).

It is a popular fermented rice-based ring-shaped spongy, pretzel-like, deep-fried snack item popular in India's Sikkim and the Darjeeling hills, as well as Nepal and Bhutan. Selroti is a Nepali word for ring-shaped rice-based bread that is traditionally cooked for religious festivals and special occasions. The main microorganisms are Lactobacilli, Pediococcus, Enterococci, and Leuconostoc (2).

Millets are minor cereals of the grass family, Poaceae. They are small seeded, annual cereal grasses, many of which are adapted to tropical and arid climates and are characterized by their ability to survive in less fertile soil (3).

They are popularly known as Nutri-cereals as they provide most of the nutrients required for the normal functioning of the human body. Finger millet was a popular crop in several Indian states. The grains were lightly roasted (occasionally after sprouting and drying), milled, and sieved. The pinkish flour (derived from red Finger millet) was consumed as a ball or gruel, sweetened or salted. Finger millet was also widely used as a weaning meal (4).

Finger millet has the highest calcium content of any cereal or millet (eight times that of pearl millet), as well as phosphorus and iron (5).

13.2 Objective:

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

13.3 Materials and Methods:

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

Methods of Preparation: The rice and Finger millet were sorted, washed, and soaked in cold water for overnight at ambient temperature. Water is then decanted from the rice, spread rice over 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.

Fermentation of the batter: After preparing the batter, 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.

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.

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.

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 (6) 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, 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).

Statistical Analysis: Each sample was analyzed in triplicates. The data obtained was analyzed statistically using standard methods given by Snedecor and Cochran (7) and by Duncan's multiple range test with the probability $p \leq 0.05$ considered to be significant (8).

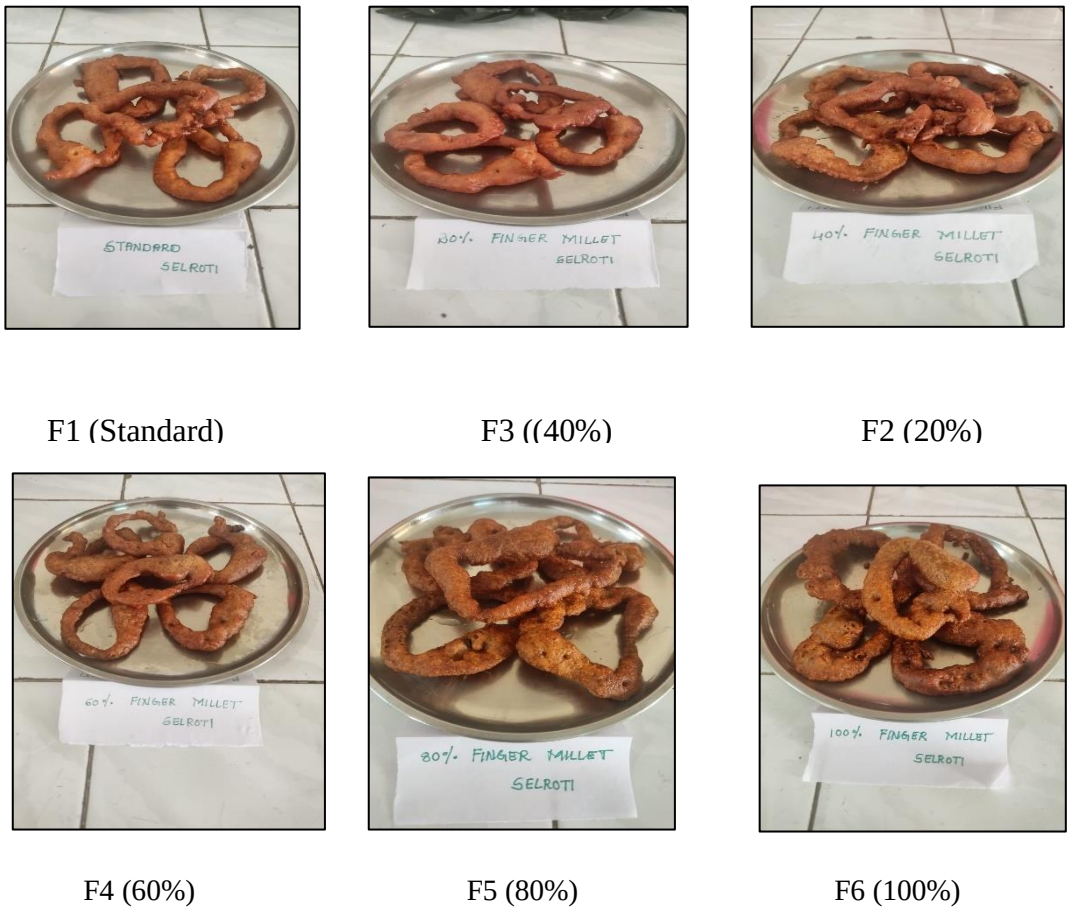
Rice + Finger millet
↓
Wash in cold water and soak for 6 hours
↓
Decant the water, pound and sieve to get flour
↓
Add refined wheat flour, jaggery and spices (cardamom, cinnamon, clove, coconut powder)
↓
Add the boiled milk to the mixture
↓
Mix thoroughly to make the batter
↓
Ferment at 28 °C for 3 hours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

Figure 13.1: Flow Chart for Preparation of Finger Millet Selroti

13.4 Formulation of the Product:

Table 13.1: Formulation of the products (ingredients g/100 g) for preparation of Finger millet Selroti

Ingredients	S1 (Standard)	S2 (20%)	S3 (40%)	S4 (60%)	S5 (80%)	S6 (100%)
Rice (g)	100	80	60	40	20	-
Finger millet (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



F1 (Standard) F3 ((40%) F2 (20%)

F4 (60%) F5 (80%) F6 (100%)

Figure 13.2: Different variations of Selroti developed from Finger millet in comparison with rice Selroti.

13.5 Result and Discussion:

13.5.1 Sensory Evaluation of Finger Millet Selroti:

The study was undertaken to prepare millet-based Selroti by partially replacing rice with Finger millet. The data pertaining to the effect of incorporation of various levels of Finger millet (20, 40, 60, 80 and 100%) on sensory attributes of Selroti and the results are shown in Table 2.

The scores obtained for all sensory attributes for F2, F3 and F4 were almost similar to the control. F5 and F6 showed decreased scores and were less acceptable compared to other variations.

The acceptable F4 variation was incubated for a different fermentation time to study the optimum fermentation time of Finger millet incorporated Selroti.

Table 13.2: Sensory evaluation of different variation of Selroti developed from Finger millet with partial replacement of Rice.

Variation	F1 (control)	F2 (20%)	F3 (40%)	F4 (60%)	F5 (80%)	F6 (100%)
Appearance	8.49±0.53	8.26±0.14	8.35±0.86	**8.46±0.23**	7.9±0.61*	7.8±0.56*
Color	8.51±0.48	8.34±0.18	8.21±0.42	**8.44±0.37**	8.01±0.67*	7.91±0.21*
Texture	8.31±0.51	8.22±0.58	8.11±0.28	**8.28±0.15**	8.1±0.08	8.24±0.23
Taste	8.55±0.69	8.38±0.11	8.40±0.77	**8.53±0.5**	7.88±0.21*	7.86±0.33*
Flavor	8.35±0.41	8.33±0.8	8.36±0.14	**8.65±0.32**	7.85±0.16*	7.12±0.28*
Overall acceptability	8.51±0.24	8.28±0.58	8.29±0.05	**8.48±0.63**	7.96±0.72*	7.67±0.48*

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

13.5.2 pH:

The 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 13.3: pH of Selroti batters.

	Initial pH	pH after 3 h	pH after 6 h	pH after 6 h	pH after 6 h
F1 (control)	6	5	5	5	5
F1 20%	6	5	5	5	5
F2 40%	6	5	5	5	5
F3 60%	6	5	5	5	5
F4 80%	6	5	5	5	5
F5 100%	6	5	5	5	5

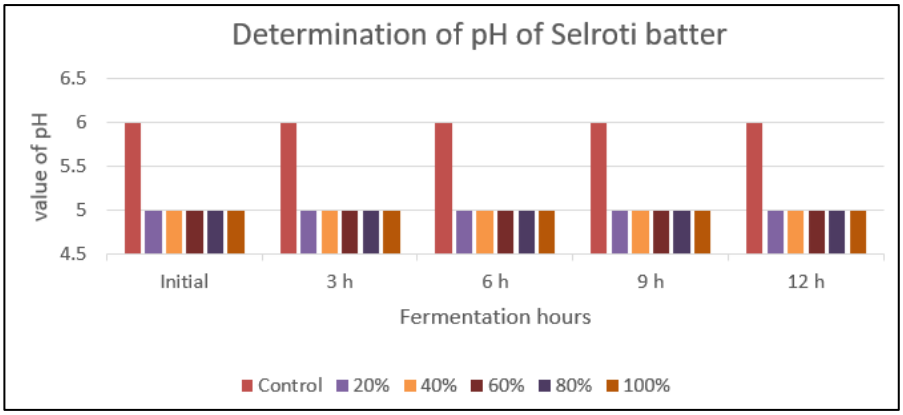


Figure 13.3: pH of Selroti Batters.

13.5.3 Volume:

The 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 13.4: Determination of Volume of Selroti Batter

	Initial Volume (ml)	Volume After 3 h (ml)	Volume After 6 h (ml)	Volume After 9 h (ml)	Volume After 12 h (ml)
F1(Standard)	80	90	90	90	90
F2	80	90	90	90	90
F3	80	90	90	90	90
F4	80	90	90	90	90
F5	80	90	90	90	90
F6	80	90	90	90	90

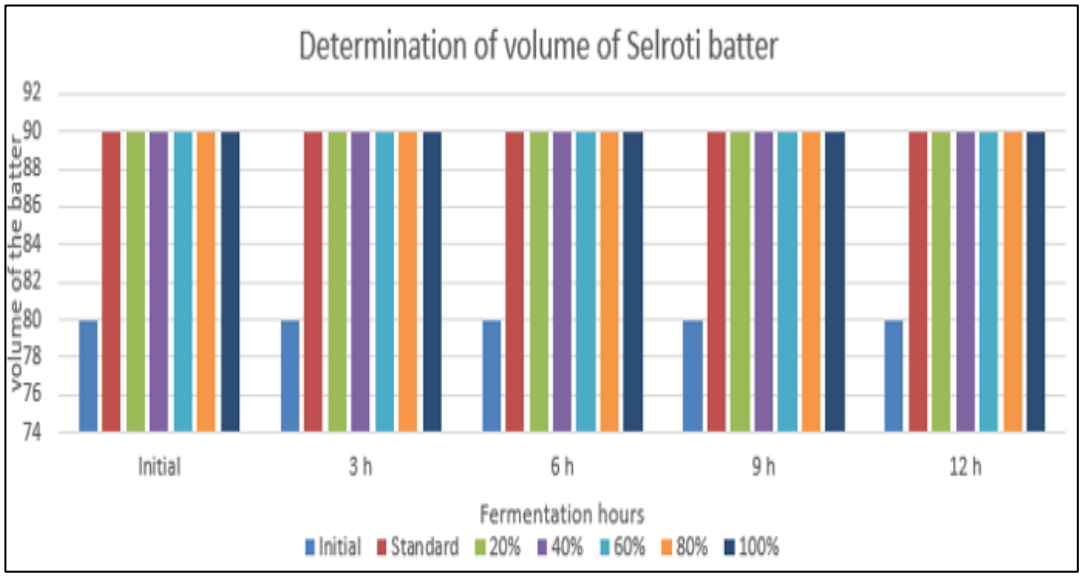


Figure 13.4: Volume of Selroti batters

13.5.4 Optimization of Batter Fermentation:

The data pertaining to the effect of incubation of different fermentation times of Finger millet (3 h, 6 h, 9 h, 12 h). The sensory attributes of Selroti and the results are shown in Table 5. The scores obtained for all sensory attributes of F1, F2, F3 and F4. The variation F3 was more acceptable in terms of sensory attributes.

Table 13.5: Sensory evaluation of different variation of Selroti developed from Finger millet with partial replacement of Rice by varying fermentation time.

Attributes	F4 (3 h)	F4 (6 h)	F4 (9 h)	F4 (12 h)
Appearance	8.46±0.23	9 ± 0*	8.65±0.26	8 ± 0*
Color	8.44±0.37	8.56 ± 0.35	8.56±0.48	8.12 ± 0.35*
Texture	8.28±0.15	8.68 ± 0.41*	7.66±0.48*	7.4 ± 0.41*
Taste	8.53±0.5	8.56 ± 0.35	7.82±0.56*	7.53 ± 0.35*
Flavor	8.65±0.32	8.46 ± 0.45	7.4±0.50*	7.26 ± 0.45*
Overall acceptability	8.48±0.63	8.8 ± 0.41	6.33±0.48*	6.56 ± 0.41*

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

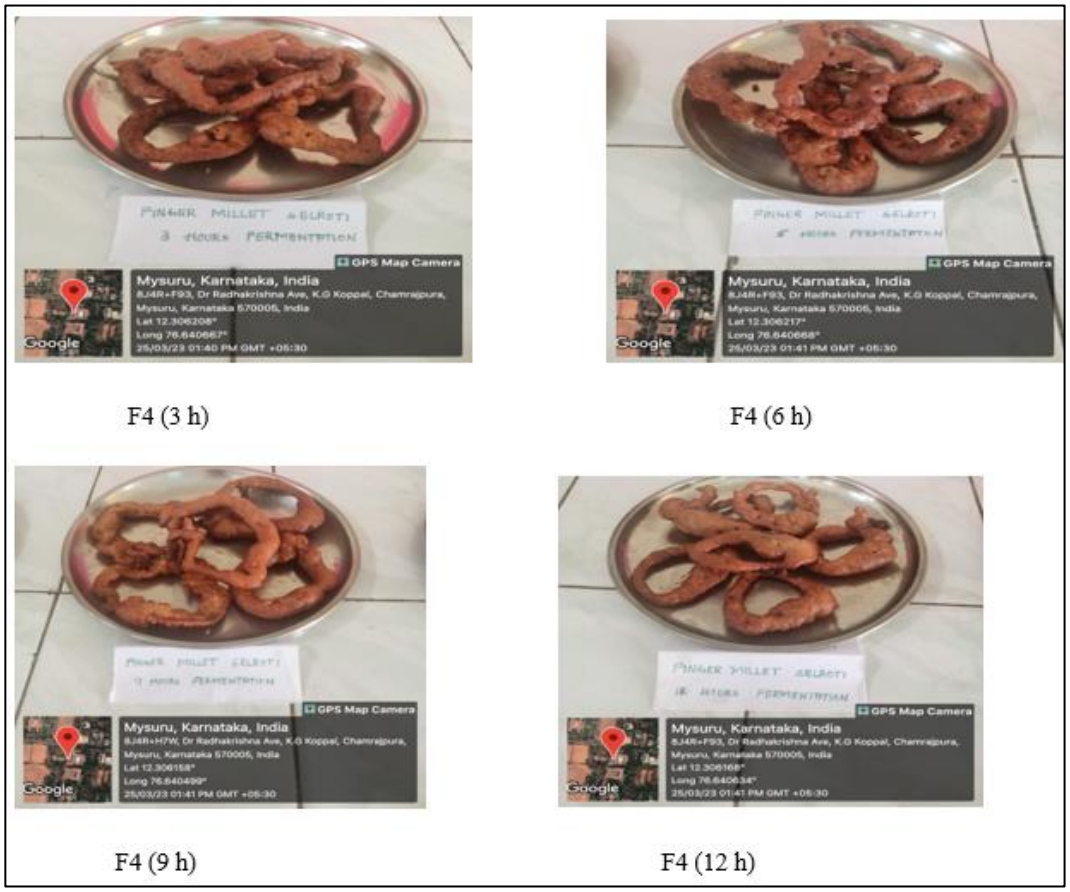


Figure 13.4: Finger Millet Incorporated Selroti Developed by Varying Fermentation Time

13.5.5 Nutritional analysis of Prepared Finger millet F4 (60%):

The proximate composition of accepted Finger millet Selroti F4 (6 h) and that of the control were analyzed and the results of the same are shown in Table 6. The moisture content of all variations of Selroti was similar. The values of protein were higher in F4 (6 h) than that of control, whereas carbohydrate was less. However, fiber, ash, iron and calcium content were increased in Finger millet Selroti.

Table 13.6: Nutritional composition of selected variation (F4) of Selroti developed from Finger millet with partial replacement of Rice.

Nutrients/100 g	Standard Selroti (F1)	F4 (6 h)
Energy (Kcal)	534.30±0.67	558±0.21*
Carbohydrate (g)	68.13±0.14	63.3±0.08*****
Protein (g)	6.91±0.08	7.2±0.12
Fat (g)	18.31±0.11	16.5±0.04*****
Fiber (g)	2.4±0.01	2.5±0.16
Ash (g)	0.42±0.03	0.5±0.17
Moisture (%)	11.39±0.25	13±0.01*****
Iron (mg)	0.28±0.02	5±0.02*****
Calcium (mg)	6.44±0.12	196±0.2*****

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

13.6 Conclusion:

With maximum scores for F4 (6h), standardising the fermentation time for a particular variety led to greater acceptance in terms of sensory qualities. In comparison to F3, Finger millet Selroti (F4), with a 60% acceptability rating, had the greatest sensory scores, while F5 (80%) and F6 (100%) had the lowest. Poor fermentation caused F4 (9 h) to have the lowest sensory scores, and hyper-fermentation caused the last variation to similarly perform poorly. Increased levels of protein, dietary fibre, iron, and calcium were found in Finger millet Selroti. Additionally, the amount of carbohydrates was decreased. The ideal fermentation period for Finger millet-infused Selroti was discovered to be 6 hours, with a 60% acceptance rate.

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14. Development of Appam from Sorghum (*Sorghum bicolor)*

Meghana A. Nayak

PG student,
Dept. of Food science and Nutrition,
Yuvaraja's college, (Autonomous),
University of Mysore,
Mysore.

Manasa R.

Research Scholar,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysore.

Shekhara Naik R.

Professor and Head,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysore.

Mahesh Shivananjappa

Assistant Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysore.

Abstract:

Appam is a traditional fermented Indian food prepared with rice flour, white sugar and coconut milk. Sorghum is one of the top five millets in India. One of the major deterrents for its use as food is the lower availability of protein, starch, and minerals due to the presence of anti-nutritional factors like tannins and phytic acid. However, processing like fermentation has proven to reduce the anti-nutritional factors, thus improving the nutritional availability and functional properties of sorghum. In this study Appam were prepared by partially replacing rice with Sorghum. Six formulations containing different composition of Sorghum flour (0%, 20%, 40%, 60%, 80% and 100%) along with white sugar and coconut milk were used to prepare Appam.

These formulations were analyzed for sensory attributes [n=20]. Appam prepared with 40% sorghum and fermented for 5 h had the highest scores in terms of sensory scores. Moreover, Appam prepared from sorghum had more protein, fiber, and phosphorus.

Keywords:

Fermentation, Anti-nutritional factors, Gluten free, Low glycaemic-index

14.1 Introduction:

Fermentation is one of the oldest and most economical methods of producing and preserving food. In addition, fermentation provides a natural way to destroy undesirable components, to enhance the nutritive value and appearance of the food, to reduce the energy required for cooking and to make a safer product.

Fermented foods are produced world-wide using various raw materials, processing methods and microorganisms. The fermented foods are part of the day-to-day diet in all parts of the world [1].

Appam is one such popular fermented breakfast food widely consumed in south India and Srilanka. It is a bowl-shaped thin pancake with a crisp edge and a fluffy centre. It is prepared with fermented batter made of rice flour, coconut milk and sugar. After the batter has fermented for 3-5 hours, it is cooked in a pan then served with some accompaniments such as spicy condiment and curry. In most of the fermented products, the fermentation is natural and involves mixed cultures of yeasts, bacteria and fungi which bring about saccharification of starch in the starting material. There are many starters traditionally used to improve the rate of fermentation like yeast, yoghurt and so on. Traditionally sugar is added to appam batter, which favours as substrate for microorganisms. Addition of sugar during fermentation, helps as the substrate for yeast, make the product soft and tender by absorbing some of the water via caramalization that helps in browning of the crust [2].

Sorghum is the fifth most important cereal in total world production. It is able to grow and produce in the warmer temperatures and tropical regions of the world. Sorghum is the chief cereal grain consumed in Asia and Africa. The protein quality of sorghum grain is poor because of the low content of essential amino acids such as lysine, tryptophan and threonine. Malting improves protein quality of cereals because of an increase in lysine [3].

Sorghum is poorly digested by infants, but if it supplemented with foods high in lysine, can be a satisfactory weaning food. The chief minerals present in sorghum grain are potassium and phosphorus, while calcium is low [4].

14.2 Objective:

- To develop millet-based appam, by partial replacement of rice by sorghum.
- To optimize its fermentation time.
- To develop low glycaemic food.

14.3 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 Rice flour, Sorghum, White sugar and Coconut milk were procured from local market of Mysuru. The Sorghum was purchased in a supermarket and sun-dried to aid in the milling and grinding process.
- **Method of preparation:** All the ingredients are mixed thoroughly to make a batter and fermented for 5 hrs. The batter is poured to hot pan and the pan is swirled. Oil is poured at the sides of the appam and it is closed by lid for 2 minutes. Then, the appam is gently removed and served.

Add all ingredients and mix thoroughly to make a homogenous batter
↓
The batter is allowed to ferment for 5 hours at room temperature
↓
After fermentation, 1 Tablespoon of fermented batter is poured to hot appam pan
↓
The pan is lifted from the stove and swirled within 3 seconds after pouring
↓
A few drops of oil were poured consistently around the appam & pan is closed by lid for 2 minutes
↓
Then, the appam is removed gently from the pan

- **Fermentation of the batter:** After preparing the batter, the batter was allowed to ferment for different periods (5, 10, 15 & 20 hours) in a stainless-steel vessel. No effort was made to control the temperature during the process of fermentation.
- **Optimization of Batter Fermentation:** For the different fermentation times and blend ratios of the Appam batter properties viz. Volume raised and pH was studied. The volume was recorded at 5, 10, 15 and 20 hours 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.
- **Sensory Analysis of Prepared Sorghum Appam:** 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 average score of the 20 semi trained panelists by using 9-point hedonic scale.
- **Nutritional analysis of Prepared Sorghum Appam:** Standard A.O.A.C. (1980) method was used to determine the Nutritional composition of selected variation (S3) of Sorghum Appam 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 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 a good precision and accuracy (5).

- **Statistical analysis:** Each sample was analysed in triplicates. The data obtained was analysed statistically using standard methods given by Snedecor and Cochran (6) and by Duncan's multiple range test with the $p \leq 0.05$ consider to be significant (7).

14.4 Formulation of the Product:

Table 14.1: Formulation of the Sorghum Appam

Ingredients	S1 (Control)	S2 (20%)	S3 (40%)	S4 (60%)	S5 (80%)	S6 (100%)
Rice flour (g)	100	80	60	40	20	-
Sorghum flour (g)	-	20	40	60	80	100
White sugar (g)	15	15	15	15	15	15
Coconut milk (ml)	50	50	50	50	50	50
Water (ml)	150	150	150	150	150	150

Figure 14.1: Different variations of Sorghum developed from Sorghum flour in comparison of Rice flour Appam.

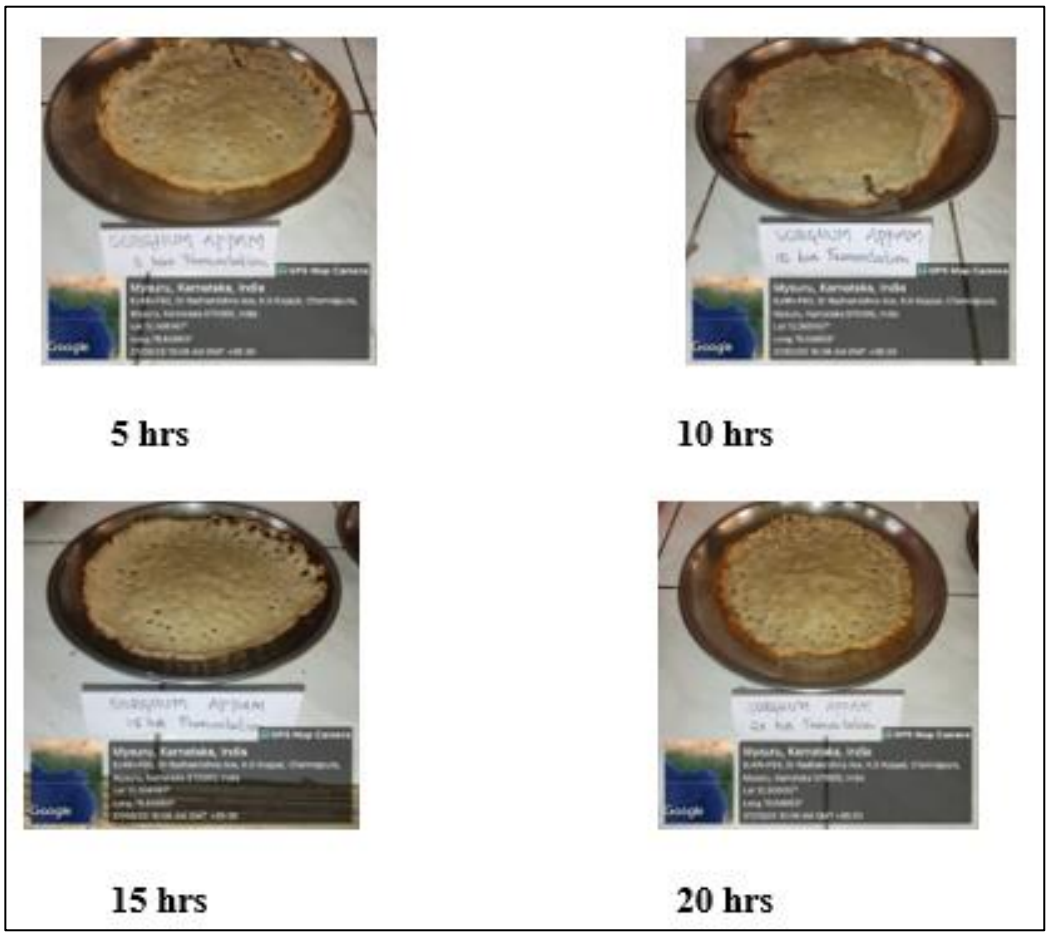

Figure 14.2: Different Variations of Sorghum Appam Developed by Varying Fermentation Time.

14.5 Results and Discussion:

The study was undertaken to prepare fiber-rich Appam by partially replacing Rice flour with Sorghum flour. They are gluten free and have low glycemic index compared to traditional Appam from rice, since they have less carbohydrates and more fiber. The data pertaining to the effect of incorporation of various levels of Sorghum flour (20, 40, 60, 80 and 100%) on sensory attributes of Appam and the results are shown in Table 2. The data pertaining to the effect of fermentation time (5, 10, 15 and 20 Hours) on sensory attributes and the results are shown in Table 3.

A. Sensory Evaluation of Sorghum Appam:

The scores obtained for all sensory attributes for S2 and S3 were almost similar on comparing with the control. Among them, S3 had higher acceptability. S5 and S6 showed decreased score and were less acceptable compared to other variations.

Table 14.2: Sensory Evaluation of Sorghum Appam

	Appearance	Color	Texture	Taste	Flavour	Overall Acceptability
S1 (control)	8.50±0.48	8.10±0.74	8.23±0.61	8.59±0.58	7.97±0.53	8.41±0.82
S2 (20%)	8.20±0.25	7.50±0.31	7.46±0.37	7.30±0.37*	7.27±0.35	7.36±0.43
S3 (40%)	8.34±0.59	7.98±0.73	7.70±0.58	8.29±0.26	7.84±0.42	7.94±0.55
S4 (60%)	8.11±0.28	7.28±0.45	7.47±0.20	8.02±0.38	7.38±0.71	7.62±0.36
S5 (80%)	7.57±0.31*	7.18±0.62	7.10±0.16*	6.83±0.54*	7.15±0.07*	7.11±0.36*
S6 (100%)	7.25±0.37*	6.97±0.21*	6.31±0.41*	6.22±0.28*	6.39±0.46*	6.74±0.15*

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

B. Sensory Evaluation of Sorghum Appam by Varying Fermentation Time:

The scores obtained for sensory attributes of different products varying fermentation time were observed. Among all the variations, Sorghum Appam of 40% which was fermented for 5 hrs had highest acceptability; whereas the same fermented for 20 hrs had least acceptability.

Table 14.3: Sensory Evaluation of Sorghum Appam by Varying Fermentation Time

	Appearance	Color	Texture	Taste	Flavour	Overall Acceptability
5 hours (standard)	8.34±0.59	7.98±0.70	7.70±0.58	8.29±0.26	7.84±0.42	7.94±0.55
10 hours	8.16±0.66	7.77±0.39	7.26±0.27	7.39±0.48*	7.47±0.39	7.38±0.56
15 hours	7.64±0.73	7.38±0.29	7.11±0.47	6.99±0.31*	7.13±0.25	7.29±0.32
20 hours	7.38±0.28*	7.29±0.42	6.85±0.24*	6.52±0.19*	6.98±0.37*	6.91±0.35*

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

C. Volume of Sorghum Appam Batter in Different Fermentation Period:

Table 14.4: Volume of Sorghum Appam Batter in Different Fermentation Period

	Initial Volume (ml)	Volume after 5 hrs (ml)	Volume after 10 hrs (ml)	Volume after 15 hrs (ml)	Volume after 20 hrs (ml)
P1 (control)	40	50	55	59	62
P2 20%	40	50	54	56	58

	Initial Volume (ml)	Volume after 5 hrs (ml)	Volume after 10 hrs (ml)	Volume after 15 hrs (ml)	Volume after 20 hrs (ml)
P3 40%	40	49	50	56	56
P4 60%	40	46	55	62	62
P5 80%	40	45	51	56	57
P6 100%	40	42	43	44	46

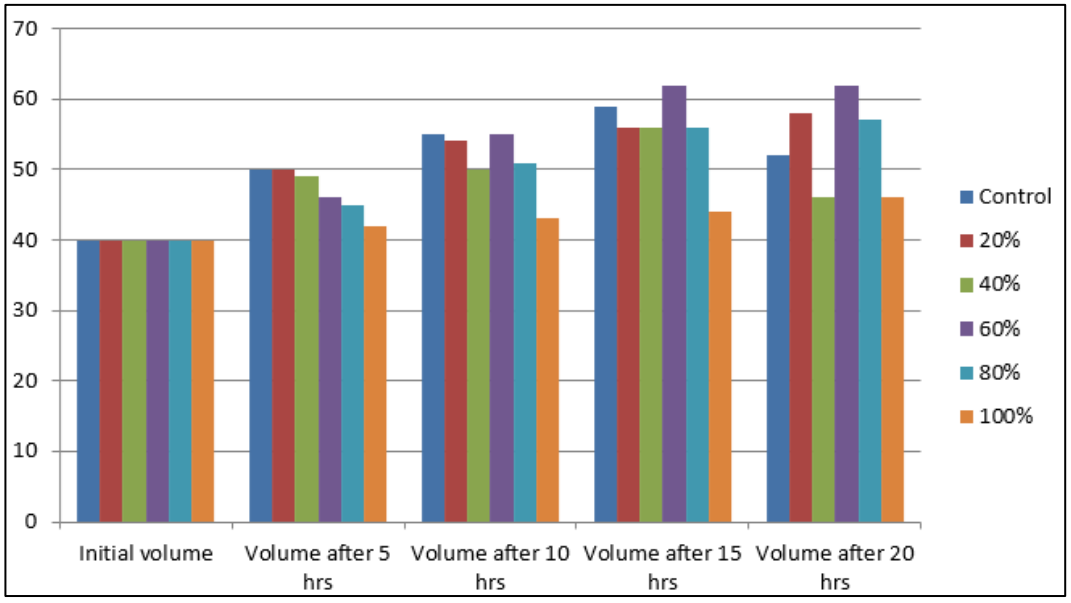

Figure 14.3: Volume of Sorghum Appam Batter in Different Fermentation Period

D. Acidity of Sorghum Batter in Different Fermentation Period:

Table 14.5: Acidity of Sorghum Batter in Different Fermentation Period

	Initial pH (ml)	pH after 5 hrs (ml)	pH after 10 hrs (ml)	pH after 15 hrs (ml)	pH after 20 hrs (ml)
P1 (control)	6.2	5.4	5.2	5.1	5.0
P2 20%	6.1	5.5	5.4	5.3	5.2
P3 40%	6.1	5.5	5.5	5.4	5.4
P4 60%	6.0	5.6	5.6	5.5	5.5
P5 80%	6.0	5.7	5.7	5.6	5.6
P6 100%	6.0	5.8	5.8	5.7	5.7

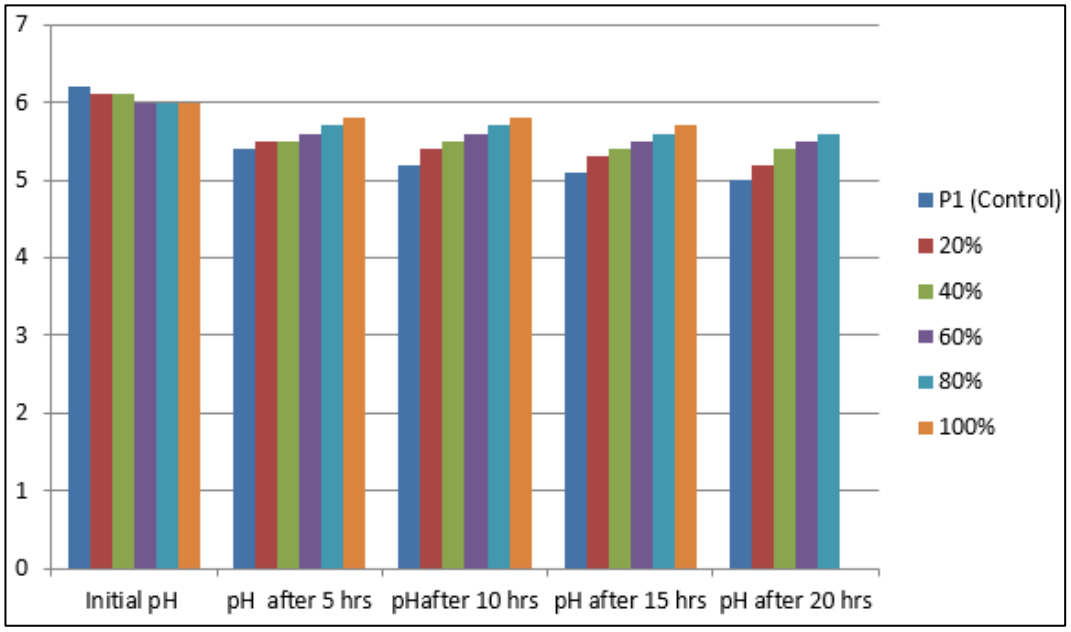

Figure 14.4: Acidity of Sorghum Batter in Different Fermentation Period

E. Nutritional Composition of Sorghum Appam:

The proximate composition of accepted Sorghum Appam (S3) and that of control were analyzed and the results of the same are shown in Table 4. The moisture content of all variations of Sorghum Appam was similar. The values of protein and fiber content were higher in S3 than that of control. However, Minerals like iron, calcium and phosphorus content were increased in Sorghum Appam.

Table 14.6: Nutritional Composition of Sorghum Appam

Nutrients (per 100 g)	Standard Appam	Sorghum Appam (40%)
Moisture (%)	36.37±0.18	38.44±0.50*
Energy (kcal)	281.24±0.63*	269.14±0.12
Carbohydrates (g)	48.15±0.28	42.07±0.43*
Protein (g)	6.95±0.12	8.79±0.32*
Fibre (g)	0.25±0.47	2.35±0.66*
Fat (g)	6.76±0.11	7.3±0.31*
Ash (g)	1.52±0.33	1.05±0.69
Iron (mg)	3.2±0.19	7.5±0.11*
Calcium (mg)	3.45±0.27	5.67±0.38*
Phosphorus (mg)	8.31±0.46	12.5±0.27*

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15. Development and Quality Evaluation of Dhokla from Kodo Millet (*Paspalum Scrobiculatum*)

Nisarga R.

PG student,
Dept. of Food science and Nutrition,
Yuvaraja's college, (Autonomous),
University of Mysore,
Mysore.

Manasa R.

Research Scholar,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysore.

Shekhara Naik R.

Professor and Head,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysore.

Mahesh Shivananjappa

Assistant Professor,
Department of Food Science and Nutrition,
Yuvaraja's College (Autonomous),
University of Mysore,
Mysore.

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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. Kodo 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 k1, k2, k3, k4, k5, k6 containing Bengal gram dhal, curd with different proportions of kodo 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 k4 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:

kodo millet, dietary fiber, fermentation, glycaemic index,

15.1 Introduction:

The word "fermentation" was derived from the Latin word "fervere," which refers to boiling. It is an enzyme catalysed and metabolic activity where the microorganisms convert sugar to alcohol and an acid anaerobically liberating energy. The science of fermentation is known as "zymology" (1). There are two main types of fermentation, known as lactic acid fermentation and alcohol fermentation. Lactic acid fermentation is a process in which sugar is converted to lactate & ATP. Where as in alcoholic fermentation sugar is converted to ethanol and ATP (1).

Fermented foods are defined as "foods or beverages manufactured by controlling the microbial growth and by converting the food constituents through enzymatic activity. Two main techniques are used to produce fermented foods. One is the food that is naturally fermented by microorganisms present in raw foods and this is called as "wild ferments" or "spontaneous ferments", another important method is by addition of starter culture called as "culture-dependent ferments" (2). Fermentation enhances the nutritional quality by reducing the anti-nutritional factors and also improves the bioavailability and digestibility of nutrients. It also improves the texture, taste, shelf life and aroma of the food (3). Fermentation also increases the mineral and trace elements bioavailability by decreasing the non-digestible plant material such as polygalacturonic and glucuronic acids, hemicellulose, cellulose and B-complex vitamins such as niacin, thiamine, folic acid and riboflavin. It also increases the iron absorption by breaking down the ferric form of iron to ferrous form with the help of vitamin C (3).

Kodo millets or magical millets are the must-have millets in your meals. Kodo millet or Paspalum scrobiculatum belongs to the family Poaceae, and is locally known as rice grass, ditch millet, cow grass in English, araka in Telugu and kodra in Marathi. Kodo millet grains are annual grains ranging from light red to dark grey. The cultivation of kodo millets started in India about 3000 years ago. Apart from India, it is cultivated in Russia, China, Africa and Japan. In India, it is widely grown in Madhya Pradesh, Tamil Nadu, Karnataka, Gujarat and Chhattisgarh. Kodo millets can be used for traditional as well as novel foods. Unprocessed or processed grain can be cooked whole or ground to flour. It can be cooked as rice and also a variety of dishes like idli, dosa, pongal, khichdi, snacks, porridge, cookies, noodles etc. (4)(5).

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) is 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 (6).

15.2 Objectives:

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

15.3 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, Mysore. The raw materials were procured from local market of Mysuru such as kodo millet, Bengal gram dhal, curd, salt, oil, turmeric powder, chillies, mustard seeds and curry leaves.
- **Method of preparation**: The standardisation of Dhokla was done by varying the proportion of kodo millet and Bengal gram dhal. The cleaned kodo 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 (7).
- **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.
- **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.
- **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.

- **Nutritional analysis of prepared Dhokla**: The proximate estimation was carried out by following standard AOAC (1990) method for chosen variation k4 (60%) & 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 (8). Calcium and iron were estimated by inductively Coupled Plasma Mass Spectrometry (ICPMS) (9).
- **Statistical analysis**: each sample was analysed in triplicates. The data obtained was analysed statistically using standard methods given by Snedecor and Cochran (10) and by Duncan's multiple range test with the $p \leq 0.05$ considered to be significant (10) (11).

15.4 Formulation of the Product:

Table 15.1: formulation of the products (ingredients g/100 g) for preparation of Kodo millet dhokla

Ingredients	Standard		20%	40%	60%	80%	100%
Kodo 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 kodo 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 15.1: Flow Chart for Preparation of Kodo Millet Dhokla

Figure 15.2: Different variations of Dhokla developed by partially replacing bengal gram dhal with kodo millet.

15.5 Result and Discussion:

A. Sensory Evaluation of Pearl Millet Dhokla:

The study was undertaken to prepare millet based Dhokla by partially replacing Bengal gram dhal with Kodo millet. The data pertaining to the effect of incorporation of various levels of kMD (20, 40, 60, 80 and 100%) on sensory attributes of Dhokla and the results are shown in Table 2. The scores obtained for all sensory attributes for k2, k3, k4, were similar to the control, whereas k5 and k6 showed decreased score and were less acceptable compared to the other variations. The acceptable k4 variation was incubated for different fermentation time to study the optimum fermentation time of kodo millet incorporated Dhokla

Table 15.2: Sensory evaluation of different variation of Dhokla developed from kodo millet with partial replacement of Bengal gram dhal.

Variation	Appearance	Colour	Texture	Taste	Flavour	Overall acceptability
K1 (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
K2 (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*
K3 (40%)	7.45 ± 0.32	7.21 ± 0.23	7.92 ± 0.12	7.12± 0.26	7.23± 0.23	7.46 ± 0.12
K4 (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*
K5 (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
K6 (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

B. pH:

Table 15.3: pH of Dhokla Batters

Variation	Initial pH	PH after 6 hrs	PH after 12 hrs	PH after 18 hrs	PH after 24 hrs
K1(control)	6	5	5	5	5
K2 20%	6	5	5	5	5
K3 40%	6	5	5	5	5
K4 60%	6	5	5	5	5
K5 80%	6	5	5	5	5
K6 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.

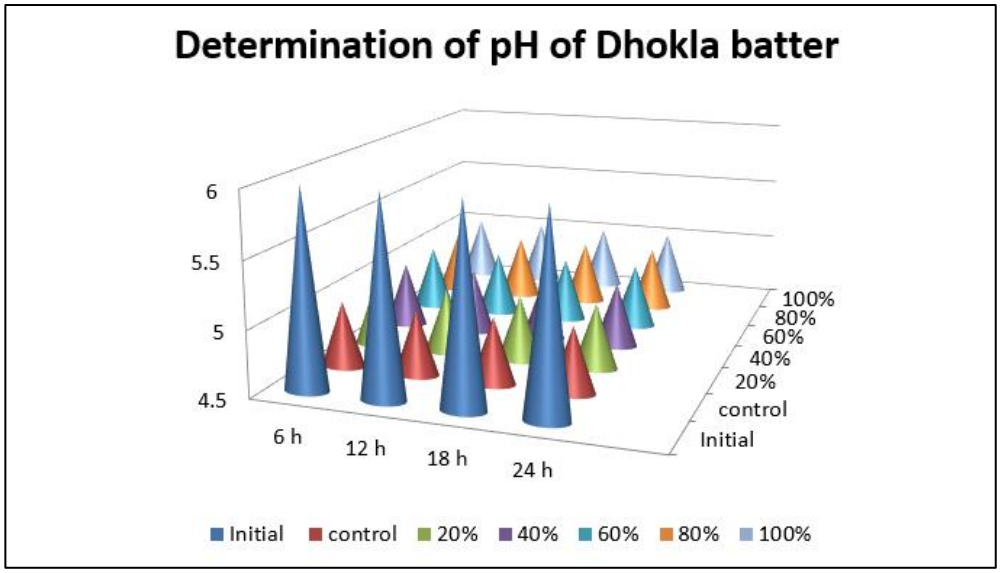

Figure 15.3:Determination of pH of Dhokla Batters

C. 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 15.4: 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)
K1 (control)	40	50	55	59	52
K2 20%	40	50	54	56	53
K3 40%	40	49	50	56	46
K4 60%	40	56	65	72	78
K5 80%	40	45	51	56	45
K6 100%	40	40	40	40	40

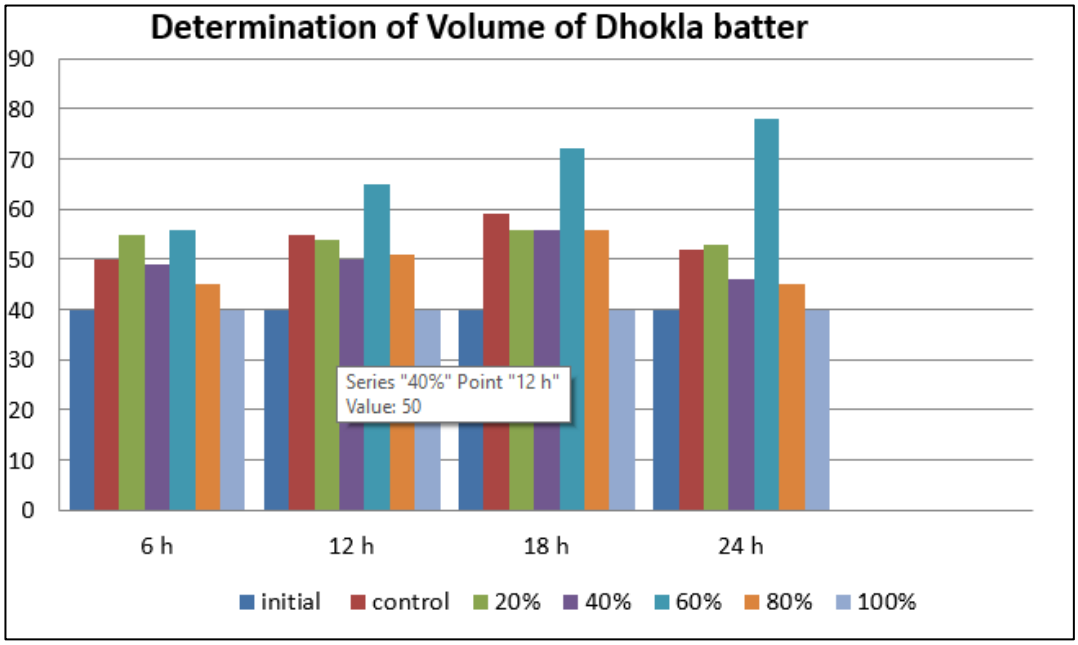

Figure 15.4: Volume of Dhokla Batters

D. Optimization of Batter Fermentation:

The data pertaining to the effect of incubation of different fermentation time of kodo millet grains (6 hrs. 12 hrs. 18 hrs. 24 hrs.). The sensory attributes of Dhokla and the results are shown in Table. The scores obtained for all sensory attributes of kM1, kM2, kM3 and kM4. The variation kM1 was more acceptable in terms of sensory attributes.

Table 15.5: Sensory evaluation of different variation of Dhokla developed from kodo millet with partial replacement of Bengal gram dhal by varying fermentation time.

Attributes	KM1 (6 h)	KM2 (12 h)	KM3 (18 h)	KM4 (24 h)
Appearance	8.51 ± 0.12*	7.51 ± 0.6	7.42 ± 0.35	6.71 ± 0.5
Colour	8.45 ± 0.16	7.23± 0.4	7.92 ± 0.23	6.42 ± 0.5
Texture	8.52 ± 0.13	7.72 ± 0.23	7.73 ± 0.9	6.23 ± 0.6
Taste	8.42 ± 0.22	7.51 ± 0.36	7.49 ± 0.9	6.31 ± 0.5
Flavour	8.61 ± 0.32	7.61 ± 0.76	7.92 ± 0.8	5.92 ± 0.5
Overall acceptability	8.92 ± 014	7.71 ± 0.32*	7.81 ± 0.9*	6.32 ± 0.5*

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

Figure 15.5: Kodo Millet Incorporated Dhokla Developed by Varying Fermentation Time

E. Nutritional Analysis of Prepared Dhokla K4 (60%):

The proximate composition of acceptable kodo millet dhokla (k4) and that of control were analyzed and the results of the same are shown in Table 4. 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 control, whereas carbohydrate was less. However, iron and Calcium content were increased in Pearl millet Dhokla.

Table 15.6: Nutritional composition of selected variation (k4) of Dhokla developed from kodo millet with partial replacement of Bengal gram dhal.

Nutrients/100 g	Control	K4 (60%)
Moisture (%)	19.06± 0.36	26.78 ± 0.31
Carbohydrates (g)	49.16± 0.15	36.45± 0.11
Protein (g)	12.18± 0.12	15.09 ± 0.21*
Fat (g)	6.32 ± 0.32	1.09 ± 0.16

Nutrients/100 g	Control	K4 (60%)
Fibre (g)	0.55± 0.10	0.71± 0.23*
Ash (g)	2.83 ± 0.02	3.41 ± 0.12
Energy (kcal)	338.8 ± 0.42	290.66± 0.33
Iron (mg)	5.33 ± 0.13	6.88± 0.13*
Calcium (mg)	44.02 ± 0.72	53.01 ± 0.22*
Phosphorous (mg)	285 ± 0.28	95 ± 0.22

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

15.6 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 k4 (6 hrs.). Kodo millet Dhokla of 60%(k4) had highest acceptability in terms of sensory scores next to k2 whereas, k5 (80%) & k6 (100%) had least acceptability. As a result of poor fermentation, kM4 (24 hrs.) had lowest sensory scores and the last variation was also not good due to hyper-fermentation. Kodo 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 kodo millet incorporated Dhokla was found to be 6 hrs. and acceptable up to 40%.

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ABOUT THE EDITORS

Dr. Talluri Rameshwari K. R., has completed her PG-Microbiology in University of Mysore and Doctoral Degree (2021) and Post-Doctoral Degree (2023) in JSS Academy of Higher Education & Research, Mysuru, Karnataka. She has 6 years of research experience, filed 2 Patents, and published 20 articles in peer reviewed journals, 05 Book chapters and 05 Books in Elsevier and UGC recognized Publishers. She as attended more than 40 National and International conferences, 95 Webinars/Seminars and presented 10 oral and 10 poster presentations among them some of them are credited with Best oral and Poster presentation awards for her innovative research work and she also credited with many awards such as "Young Microbiologist Award - 2018", "SERS-Outstanding Research Student Award - 2021", for the best research paper presentation in the international conference held in Dehradun, Uttarakhand, "Young Women Scientist Award - 2022", and "Young Scientist Award for Life Sciences – 2022" in the various International conferences in India. She has obtained 'Second Ranked' in MA in English (2021) and also Completed her Master of Social Work (MSW) in Medical Psychiatry, Social and Women Welfare as her main specialization (2023) from the Sri Venkateshwara University, Director of Distance Education, Tirupati, Andhra Pradesh. She was awarded with Karnataka State Government Fellowship for Merit Student in (2017-19), ICMR-Senior Research Fellowship in (2019-21) and DST-Biomedical Device Technology Development (BDTD), (2021-23), Government of India, New Delhi for the Development of Device for the detection of Mycobacterium tuberculosis. She is also Active Committee Member and Research Team member in "The Society for Green Environment (SGE)", Life time member in "Agro-Environmental Development Society (AEDS)", and "Association of Microbiologists of India (AMI)" And also General Secretary and Director of "Microbiological Association for Science and Technology Development (MASTD)" which is a multi-disciplinary membership based-national organization of individuals, institutions, and corporations, Government of India, New Delhi. Her Major theme of research Specialization is on Epidemiological studies, Histopathology, Molecular Biology, Biochemistry, Cell Biology, Bioinformatics etc.,

Dr. Kshitij Singh is currently Joint Secretary in the Microbiological Association for Science and Technology Development (MASTD), registered under the Society Registration Act XXVI, 1860 in the state of Uttar Pradesh. His doctorate is in the subject of Environmental Microbiology from the Babasaheb Bhimrao Ambedkar University, Lucknow. His Masters was in Plant Biotechnology from the Banaras Hindu University, Varanasi. He has published several review and research paper in high impact factor journal. Moreover, he has also published one international patent. He is also the reviewer in the several journals of Springer and Elsevier.

Dr. Ajay Kumar Singh is currently working as Research Fellow in Ministry of Environment, Forest and Climate Change Department, Uttar Pradesh. Additionally, he is also the President of Microbiological Association for Science and Technology Development (MASTD), registered under the Society Registration Act XXVI, 1860 in the state of Uttar Pradesh. Moreover, he has also organized several National and International Conference in the field of Life Sciences. His doctorate is in the subject of Environmental Microbiology from the Babasaheb Bhimrao Ambedkar University, Lucknow. He has published several research and review articles in High Impact Factor, International journals in the relevant field. He is also the reviewer in the several journals of Elsevier.

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