

**STANDARDIZATION OF PROCESS PARAMETERS FOR IDLI BATTER
FERMENTATION USING SELECTED STARTER CULTURE
COMBINATIONS**

By,

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KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF PROCESSING AND FOOD ENGINEERING

**KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND FOOD
TECHNOLOGY**

TAVANUR-679573, MALAPPURAM

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PROJECT REPORT

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In

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KERALA AGRICULTURAL UNIVERSITY

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2026

DECLARATION

We hereby declare that this project report entitled “**STANDARDIZATION OF PROCESS PARAMETERS FOR IDLI BATTER FERMENTATION USING SELECTED STARTER CULTURE COMBINATIONS**” is a bonafide record or research work done by us during the course of research and that the report has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Place: Tavanur

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CERTIFICATE

Certified that this project report entitled “**STANDARDIZATION OF PROCESS PARAMETERS FOR IDLI BATTER FERMENTATION USING SELECTED STARTER CULTURE COMBINATIONS**” is a bonafide record of research work done by MEERA KRISHNA P. M. (2022-06-023), ANSHITHA ISMAIL (2022-06-024), HANNA THESLIN K. C. (2022-06-025), NITHYA K (2022-06-027), and FATHIMA GHILSHA P (2022-06-030) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to them.

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SYMBOLS AND ABBREVIATIONS

%	: Percentage
&	: And
/	: Per
+	: Plus
=	: Equal to
±	: Plus or minus
°C	: Degree Celsius
3D	: Three-Dimensional
a*	: Greenness or redness
ANOVA	: Analysis of variance
aw	: Water activity
b*	: Blueness or yellowness
cm	: Centimetre
et al.	: And others
etc	: Etcetera
Fig	: Figure
g	: Gram

min	: Minutes
i.e	: That is
KAU	: Kerala Agricultural University
KCAEFT	: Kelappaji College of Agricultural Engineering and Food Technology
W	: Watt
L*	: Lightness or darkness
N	: Normality
rpm	: Rotation per minute
RSM	: Response surface methodology
viz.,	: Namely
mm	: Millimetre
mL	: Millilitre
m ³	: cubic metre
MHz	: Megahertz
kHz	: Kilo hertz
cm ³	: Cubic centimetre
µg	: Microgram
TPC	: Total Plate Count

**DEDICATED TO FOOD TECHNOLOGY
PROFESSION**

INTRODUCTION

CHAPTER I

INTRODUCTION

Idli is one of the most popular traditional fermented foods from South India, enjoyed as a staple breakfast and dinner for centuries. It is made from fermented batter that mainly consists of rice (*Oryza sativa*) and dehulled black gram (*Vigna mungo*), typically mixed in ratios ranging from 2:1 to 4:1. People appreciate idli for its soft texture, pleasant taste, ease of digestion, and nutritional value, making it suitable for all age groups. Historical evidence indicates that idli originated in South India over a thousand years ago, with references to similar foods appearing in ancient texts like *Vaddaradhane* (920 AD). Over time, it evolved from a basic steamed cereal dish to the fermented rice-legume product we know today, becoming a key part of the culinary traditions in Kerala, Tamil Nadu, Karnataka, and Andhra Pradesh. In recent years, idli has gained recognition beyond India for its health benefits and status as a nutritious fermented food.

The nutritional value of idli mainly comes from the combination of rice and black gram. Rice is the main source of carbohydrates, while black gram provides proteins, minerals, and functional constituents that improve the batter's quality and texture. A typical serving of idli offers about 140–160 kcal of energy, 28–32 g of carbohydrates, and 4–6 g of protein, along with minerals like calcium, iron, phosphorus, potassium, and magnesium. Fermentation enhances the nutritional profile by increasing the availability of B-complex vitamins, improving mineral absorption, and making it easier to digest through the partial breakdown of starches and proteins. Because of its low fat content, cholesterol-free nature, and easy digestibility, idli is considered as a nutritious food for children, the elderly, and health-conscious individuals.

Idli's quality develops during fermentation, a natural process mainly carried out by lactic acid bacteria (LAB) and yeasts. During this process, microorganisms convert carbohydrates into organic acids, carbon dioxide, and other metabolites that influence flavour, texture, and preservation. LAB produce lactic acid, which lowers the pH from about 6.5 to 4.5–5.0. Meanwhile, yeasts create carbon dioxide, causing the batter to expand and aerate. The combined action of these microorganisms results in the soft, porous, and spongy texture typical of high-quality idli. Fermentation also triggers several biochemical changes, including starch breakdown, lactic acid fermentation, alcoholic fermentation, protein degradation, and interactions between acid and gas.

These changes lead to better flavour, higher digestibility, improved nutritional value, and enhanced sensory quality.

Scientific studies on idli fermentation have highlighted the role of microbial interactions in determining the batter quality. Early research showed that naturally occurring LAB and yeasts work together to manage acid production, gas formation, and flavour development. LAB populations can reach 10^7 – 10^8 CFU/g during vigorous fermentation, while yeasts significantly contribute to the batter's leavening and volume increase. Studies on optimizing starter cultures reported improvements in batter volume by up to 28%, reductions in pH from 6.5 to 4.8, and sensory acceptability scores above 8.5 on a 9-point scale. Other research has shown improvements in protein digestibility, better mineral absorption, and enhanced microbial stability with selected starter cultures. Recent findings also indicated that combinations of LAB and yeast could extend the refrigerated shelf life of idli batter from 2 days to about 7 days while maintaining good sensory quality.

Despite its popularity, traditional idli fermentation heavily relies on natural micro-flora and local environmental conditions. Variations in temperature, humidity, quality of raw materials, grinding methods, water content, and microbial populations often lead to inconsistent fermentation results. Such inconsistencies can result in excessive sourness, poor rise, undesirable texture, unstable acidity, and reduced shelf life. Batch-to-batch variations in flavour, softness, and overall quality are common, especially in commercial production. These issues worsen with the growing demand for ready-to-use and refrigerated idli batter products, which need consistent quality and longer shelf stability. Traditional spontaneous fermentation often fails to meet these demands since microbial growth and acid production are mostly uncontrollable.

The rapid growth of the ready-to-cook and refrigerated idli batter market, fueled by urbanization, changing lifestyles, and a greater preference for convenience, has created a strong demand for fermentation systems that ensure product quality throughout processing, distribution, and storage. However, manufacturers face a major challenge with excessive acid build-up after fermentation due to continued microbial activity during refrigeration. This can lead to unwanted sourness, flavour loss, separation, and changes in batter consistency, which ultimately reduce consumer appeal and shorten shelf life. Commercial batters often remain acceptable for only one to two days in the fridge before significant quality declines. Therefore, managing microbial activity

and controlling acid production during storage have become crucial for the successful commercial production of idli batter.

Using selected starter cultures has emerged as a promising solution to these challenges. Starter cultures help create controlled fermentation by ensuring predictable growth of microorganisms, acid production, and gas formation. Controlled fermentation allows for better regulation of pH, acidity, rise, texture, and flavour, leading to more consistent products with longer shelf lives. Selected LAB and yeast cultures can also inhibit unwanted microorganisms, decrease spoilage, and boost consumer acceptance. Moreover, starter cultures offer a chance to standardize fermentation processes, which is vital for large-scale commercial production. Recent advances in starter culture technology have led to the creation of specific microbial combinations that can provide reliable fermentation performance while maintaining the traditional sensory qualities of fermented foods.

Although many studies have looked at starter culture fermentation in idli batter, most focus on improving fermentation efficiency, batter volume, and texture. There is limited information on optimizing specific starter culture combinations to control acid levels, reduce excessive sourness, and improve storage stability in refrigeration. Thus, it is necessary to evaluate selected microbial combinations and fermentation factors systematically to develop a standardized process for producing high-quality idli batter with desirable sensory characteristics and longer shelf life. Considering these factors, this study titled “STANDARDIZATION OF PROCESS PARAMETERS FOR IDLI BATTER FERMENTATION USING SELECTED STARTER CULTURE COMBINATIONS” was conducted at the Department of Processing and Food Engineering, Kelappaji College of Agricultural Engineering and Food Technology, Tavanur. The study aimed to:

- (i) To optimize and standardize the process parameters for idli fermentation using selected starter culture combinations
- (ii) To evaluate the physico-chemical changes occurring in starter culture based idli batter during fermentation
- (iii) To evaluate the shelf life and sensory attributes of optimized idli batter under refrigerated storage conditions

The findings of this study are expected to provide a scientific basis for the standardization of a starter culture-based fermentation process for idli batter, enabling improved process

consistency, controlled fermentation and acidification, and enhanced shelf-life stability during refrigerated storage, thereby supporting the production of high-quality commercial idli batter.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

This chapter presents a comprehensive review of the existing research related to idli batter and its fermentation process, with particular emphasis on the standardization of process parameters for idli batter fermentation using starter cultures. Idli, a traditional fermented cereal-legume food, is widely recognized for its nutritional value, digestibility, and sensory appeal. The quality of idli batter is influenced by several factors, including ingredient composition, fermentation conditions, microbial activity, and the use of suitable starter cultures. This review examines previous studies on ingredient optimization, fermentation dynamics, physico-chemical and sensory characteristics, microbial quality, and methods employed to enhance the consistency and shelf life of idli batter. It also discusses the role of selected starter cultures in improving fermentation efficiency, product quality, and process standardization. Finally, the chapter identifies the existing research gaps in the development of standardized starter culture-based idli batter, providing the scientific basis and rationale for the present investigation.

2.1 IDLI

Idli is a traditional South Indian savory rice cake, widely popular as a breakfast food across Southern India and Sri Lanka, and now enjoyed throughout the country for its lightness, digestibility, and nutritional benefits. It is made by steaming a fermented batter of rice and de-hulled split black gram (urad dal). It is a nutritious, gluten-free food that forms an important part of daily meals and restaurant menus.

The process of idli fermentation is traditionally driven by natural micro flora from ingredients or the environment. However, the standardization of idli batter using defined starter cultures has gained importance due to demands for consistency in texture, flavour, nutritional quality, and shelf life in commercial and domestic settings. Recent years have seen significant research aimed at optimizing starter cultures for idli batter and understanding their impact on fermentation dynamics and end-product characteristics. The review highlights conventional fermentation and modern fermentation techniques using starter culture, emphasizing the advantages of fermentation using starter culture, additionally, the role of each microorganism in the fermentation process. This was concluded by identifying a clear research gap in optimized use of each starter culture.

The batter undergoes overnight fermentation, during which naturally occurring microorganisms such as *Leuconostoc mesenteroides* and *Streptococcus faecalis*, present on grains, legumes, or utensils, multiply rapidly and outcompete initial contaminants, thereby dominating the process (Mukherjee *et al.*, 1965). These microbes produce more than 1.0% lactic acid along with carbon dioxide, which establishes anaerobic conditions in the batter and contributes to leavening (Mukherjee *et al.*, 1965). Several factors, including the type of raw materials, fermentation or processing temperature, and the microorganisms involved, bring about biochemical and nutritional transformations.

Different authors have reviewed methods of idli preparation (Soni and Sandhu, 1990; Joseph *et al.*, 1993). Fermentation has also been reported to enhance the levels of vitamins B and C while leading to approximately 50% hydrolysis of phytate (Rajalakshmi and Vanaja, 1967). (Mukherjee *et al.*, 1965) observed that *L.mesenteroides* (responsible for leavening) and *Enterococcus faecalis* (responsible for acid production) start proliferating together during soaking and continue to multiply after grinding. Similarly, Marfo *et al.*, 1990 noted that fermentation of rice and legume batter decreases phytate by 45% after one day, 75% after two days, and 80% after three days.

Several researchers have further documented that methionine content rises from 10.6% to 60.0% during fermentation (Rao *et al.*, 1961; Rajalakshmi and Vanaja, 1967; Steinkraus *et al.*, 1967). This increase in methionine, which is otherwise a limiting essential amino acid in legumes, substantially improves the overall protein quality of the product.

2.2 IDLI BATTER

Idli batter is a naturally fermented mixture used to prepare the iconic South Indian breakfast, idli. The quality of the batter is fundamental for producing soft, fluffy, and flavourful idli cakes. It is made by steaming a naturally fermented batter of rice and de-husked black gram (urad dal), resulting in soft, spongy, mildly sour cakes that are easy to digest and rich in protein and carbohydrates. The batter is prepared by different steps such as soaking, grinding and resting to allow fermentation. Consistency of the final batter should be thick but pourable, not runny or very stiff.

2.2.1 RAW MATERIALS

The raw materials used in this study included polished parboiled rice (*Oryza sativa*, Mahamaya, IET-10749 variety), commercially known as Kranthi, and decorticated black gram (*Vigna mungo*) both of which were procured from local market.

2.3 IDLI PREPARATION PROCESS AND ROLE OF INGREDIENTS

The main ingredients of idli are rice, black gram, salt, and water. The preparation of idli batter (Fig 2.1) involves several steps. Rice and black gram are first soaked in water for 5–10 hours. After draining the water, both are ground separately in a wet mill with intermittent addition of water. The two batters are then combined in a vessel, salt is added, and the mixture is allowed to ferment overnight at room temperature. On the following day, the fermented batter is steamed in an idli steamer for about 10 minutes before consumption (Steinkraus *et al.*, 1983; Soni *et al.*, 1986; Nisha *et al.*, 2005).

The quality of idli depends greatly on the type and proportion of rice and black gram used. Kumar *et al.*, 2005 showed that different rice cultivars (Jaya and Minilong) and varying polishing levels (high vs. low) produced noticeable differences in fermentation behavior and sensory quality. Previous studies tested rice-to-black gram ratios ranging from 2:1 to 4:1, but higher rice proportions were less preferred because of the dominant rice flavour (Steinkraus, 1996). Ratios of 2:1 (Reddy *et al.*, 1982) and 3:1 (Sridevi *et al.*, 2010) were found to give better quality idli with improved sensory properties.

Black gram plays a crucial role in idli preparation as it contributes to the foamy consistency of the batter and helps retain gases during fermentation (Reddy *et al.*, 1982). Its surface-active proteins (globulins) and arabinogalactans are responsible for the spongy texture of steamed idli (Nisha *et al.*, 2005). Substitutes such as soybean, chickpea, and green gram have been attempted (Steinkraus, 1996), but black gram remains the preferred choice due to its superior sensory characteristics. Although substitutions may increase nutritional value, consumer acceptance is largely influenced by texture and flavour.

Salt also plays an important role by enhancing taste and overall acceptability (Soni & Sandhu, 1989). Previous studies have used salt concentrations between 0.85% and 2% (w/v). Water is added at 1.1–2.5 times the weight of the dry ingredients, depending on the consistency required for batter preparation (Reddy *et al.*, 1982).

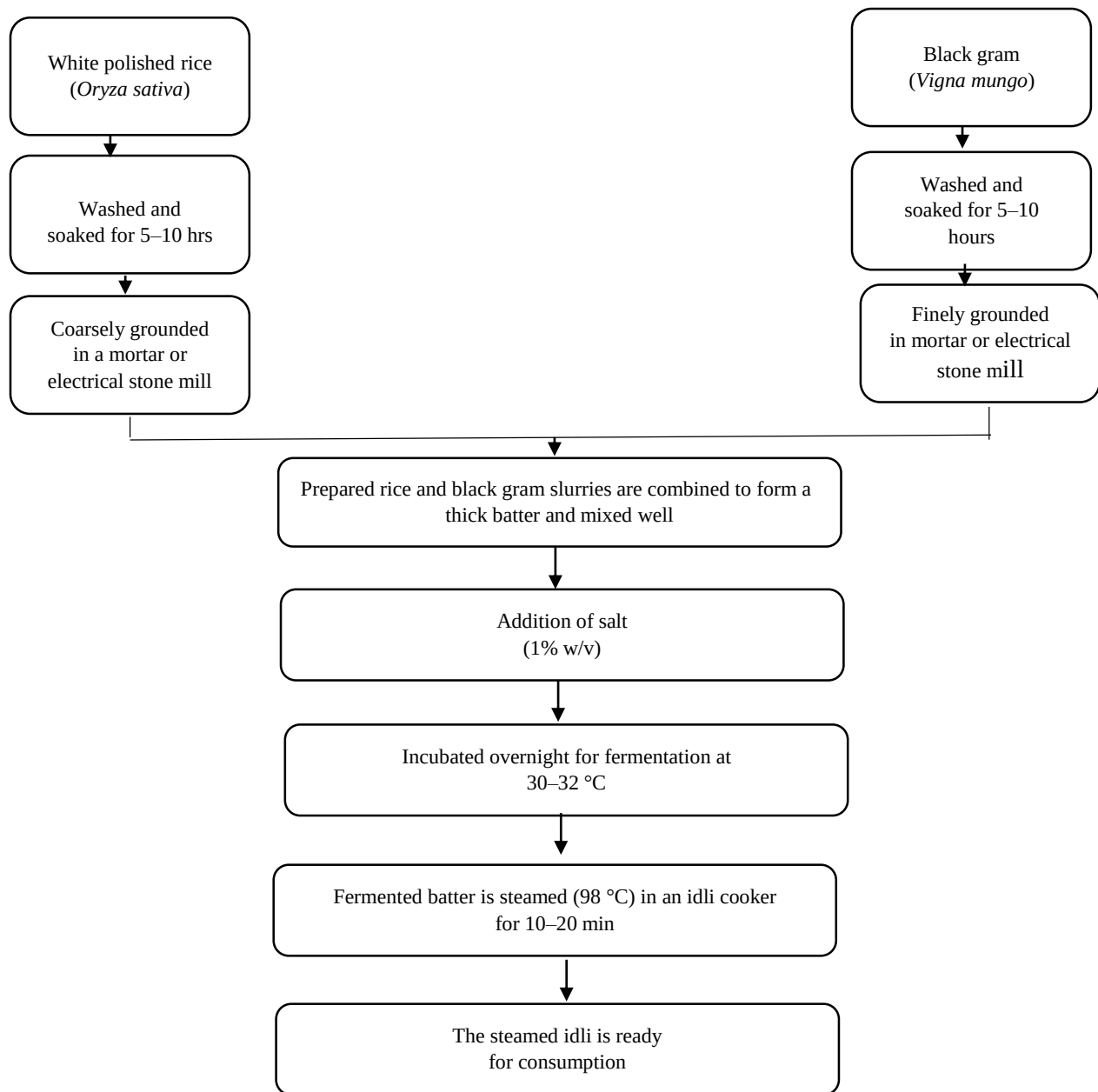


Fig 2. 1: Steps involved in the preparation of idli (Steinkraus 1983; Murthy and Rao 1997; Nisha *et al.*, 2005)

2.4 DETERMINATION OF IDLI BATTER PROPERTIES

The properties of idli batter were evaluated at different fermentation times and blend ratios. The parameters assessed included bulk density, pH, total acidity, flow behaviour index, and

consistency coefficient. Bulk density was determined as the ratio of batter mass to volume. The pH of the batter was measured using an ORLAB pH meter (ORLAB Instruments Pvt. Ltd., Hyderabad, India). Total acidity was determined by diluting 5 g of fresh batter with 10 mL of distilled water, followed by titration against 0.1 N NaOH, with results expressed as percentage acidity.

Rheological characteristics, namely flow behaviour index and consistency coefficient, were measured using a rotational viscometer (Visco Basic, GR Scientific Ltd., Bedfordshire, UK) according to ISO 2555 and ISO 1652 standards. The viscometer was operated within a speed range of 5–100 rpm using spindle type L4 at 30 ± 1 °C. Since direct shear rate values are not provided for spindle geometry, they were calculated from torque and spindle speed following the methods of (Mitschka *et al.*, 1982) and (Nagaraju and Manohar 2000). The flow behavior index (n) and consistency coefficient (k) were then obtained using the power law model by plotting shear stress against shear rate on a log–log scale (Balasubramanian *et al.*, 2006).

2.5 MICROBIOLOGY OF IDLI

Idli continues to be a widely popular food in India, largely due to the distinctive texture and flavour that develop during fermentation (Nisha *et al.*, 2005). The fermentation process is naturally dominated by indigenous lactic acid bacteria (LAB) and yeasts, with the specific microflora often determined by the ingredients used and the surrounding environment (Desikachar *et al.*, 1960). Factors such as fermentation temperature and seasonal variation also influence microbial succession. For example, LAB and yeast populations are generally higher in winter, while yeast counts tend to decline during summer, possibly due to elevated temperatures (Soni *et al.*, 1986).

LAB and yeast begin to develop during the soaking of rice and black gram (Mukherjee *et al.*, 1965 and Soni *et al.*, 1986), with each ingredient contributing distinct species (Soni *et al.*, 1986). Numerous LAB and yeast species have been identified as participants in idli fermentation (Table 2). In the absence of soaking, however, an external inoculum is required to achieve effective fermentation (Reddy *et al.*, 1982). Alongside the dominant organisms, other microorganisms such as *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Paenibacillus polymyxa*, *Enterobacter spp.*, and *Kocuria varians* (Soni *et al.*, 1986). This indicates that the microbial community in idli fermentation is heterogeneous (Table 2).

The dominant organisms develop sequentially, beginning during soaking and continuing into batter fermentation. Freshly fermented batter typically contains high populations of LAB and yeast, ranging from 10^8 – 10^{11} cfu/g (Mukherjee *et al.*, 1965; Soni *et al.*, 1986; Nisha *et al.*, 2005; Sridevi *et al.*, 2010; Iyer *et al.*, 2011).

Fermentation of idli brings about two key changes: acidification and leavening of the batter. During fermentation, microbial counts rise, accompanied by a decrease in pH due to increasing acidity. After 12–18 hours of fermentation at room temperature (30°C), lactic acid levels in the batter reach approximately 1.2–2.5 g, with a final pH around 4.5 (Mukherjee *et al.*, 1965). At the same time, the batter expands in volume as a result of carbon dioxide production by the microorganisms (Steinkraus, 1996).

LAB/ yeasts	References
<i>Lueconostoc mesenteroides</i>	Mukherjee et al. (1965)
<i>Streptococcus faecalis</i>	Mukherjee et al. (1965)
<i>Pediococcus cerevisiae</i>	Mukherjee et al. (1965)
<i>Lactobacillus delbrueckii</i>	Soni et al. (1986)
<i>Lb. fermentum</i>	Soni et al. (1986)
<i>Lactococcus lactis</i>	Iyer et al. (2011)
<i>Lb. plantarum</i>	Iyer et al. (2011)
<i>Saccharomyces cerevisiae</i>	Soni et al. (1986)
<i>Torulopsis candida</i>	Reddy et al. (1982)
<i>Trichosporan pullulans</i>	Batra and Millner (1974)
<i>Trichosporon beigelli</i>	Soni et al. (1986)
<i>Hansenula anomala</i>	Soni et al. (1986)
<i>Oosporidium margaritferium</i>	Soni et al. (1986)
<i>Candida robusta</i>	Soni et al. (1986)
<i>Kluyveromyces marxianus</i>	Soni et al. (1986)
<i>Candida kefir</i>	Soni et al. (1986)
<i>Candida krusei</i>	Soni et al. (1986)
<i>Rhodotorula mucilaginosa</i>	Soni et al. (1986)
<i>Rhodotorula glutinis</i>	Soni et al. (1986)

Fig 2.2: Microorganism involved in idli fermentation (from Soni *et al.*, 1986)

Although different microorganisms participate in idli fermentation, only certain species are capable of producing both acid and gas. In one study, 290 bacterial strains were isolated from idli batter at different fermentation intervals. Among them, 127 strains produced both acid and gas, while the rest produced only acid. During the early stages of fermentation, *Leuconostoc mesenteroides* and *Enterococcus faecalis*, both low acid producers, were dominant.

In the later stages, *Pediococcus cerevisiae* was more prevalent. The authors concluded that the heterofermentative *L. mesenteroides* was the dominant organism throughout fermentation and the key contributor to leavening. Interestingly, no yeast activity was observed in this study (Mukherjee *et al.*, 1965). The importance of *L. mesenteroides* in leavening was further confirmed by inoculating pure cultures of LAB, isolated from idli, into autoclaved ingredients. Results showed that both *L. mesenteroides* and *Lactobacillus fermentum* played a role in leavening (Ramakrishnan, 1979).

The role of yeast in idli fermentation, however, has been more complex and somewhat controversial. While Mukherjee *et al.* (1965) reported that only LAB were responsible for leavening, later studies demonstrated that yeasts also play an equally important role (Soni *et al.*, 1986; Sridevi *et al.*, 2010). Several yeast species have since been identified from idli fermentation, which is not surprising given their ability to thrive in low pH environments. Biochemical studies revealed that the predominant yeasts not only produced gas and utilized sugars alongside LAB, but also secreted amylases—enzymes absent in LAB strains (Soni *et al.*, 1986). Based on these findings, Venkatsubbaiah *et al.*, (1984) proposed that LAB reduce the pH of the batter by producing acids, thereby creating favorable conditions for yeast activity. In return, yeasts support LAB growth by breaking down starch through their extracellular amylolytic enzymes. However, the precise nature of this synergistic relationship between LAB and yeasts still requires further clarification.

Traditionally, microorganisms involved in idli fermentation have been studied using culture-based methods. LAB counts are usually determined on MRS agar, while yeasts are enumerated on potato dextrose agar (Rekha & Vijayalakshmi, 2011) or Sabouraud dextrose agar (Sridevi *et al.*, 2010). Aerobic and mesophilic organisms are counted on tryptone glucose yeast extract agar (Mukherjee *et al.*, 1965) or nutrient agar (Rekha and Vijayalakshmi, 2011). Despite these advances, modern molecular tools for identification and typing are still underutilized, and could provide a more comprehensive understanding of the microbial dynamics at each stage of fermentation.

2.6 TRADITIONAL IDLI FERMENTATION

Idli is a traditional South Indian fermented food, fermentation relies on spontaneous, naturally occurring microflora, mainly from grains and the environment. Traditionally, idli preparation is as follows: i) rice and black gram were soaked separately, ii) after draining the water, rice and black gram were grind separately with occasional addition of water during grinding

process, iii) the rice and black gram batters were then mixed together with addition of a little salt, iv) the mixture was allowed to ferment overnight at room temperature, v) the fermented batter was dispensed in special idli pans and allowed for steaming for 5–8 min (Balasubramanium *et al.*, 2006). During fermentation of idli batter overnight the naturally occurring microorganisms viz. *Leuconostoc mesenteroides* and *Streptococcus thermophilus* in grains/legumes/utensils grow rapidly, outnumbering the initial contaminants and dominating the fermentation. These microorganisms produce lactic acid ($\geq 1.0\%$) and carbon dioxide that make the batter anaerobic and leaven the product. Several aspects such as effect of raw materials, effect of fermentation or processing temperature and microorganisms involved in biochemical and nutritive changes have been investigated (Balasubramanium *et al.*, 2006, Desikachar *et al.*, 1962, Joseph *et al.*, 1993, Steinkraus *et al.*, 1967, Steinkraus *et al.*, 1983, Thyagaraja *et al.*, 1991). Methods of idli preparation have been reviewed by many authors (Soni and Sadhu 1990). It has also been reported that during fermentation, vitamins B and C increase, and also phytate is hydrolyzed almost to 50%. *L. mesenteroides* (leavening) and *S. faecalis* (acid production) develop concomitantly at soaking stage and continue to multiply following grinding (Mukherjee *et al.*, 1965).

2.7 NUTRITIONAL AND CHEMICAL COMPOSITION OF IDLI

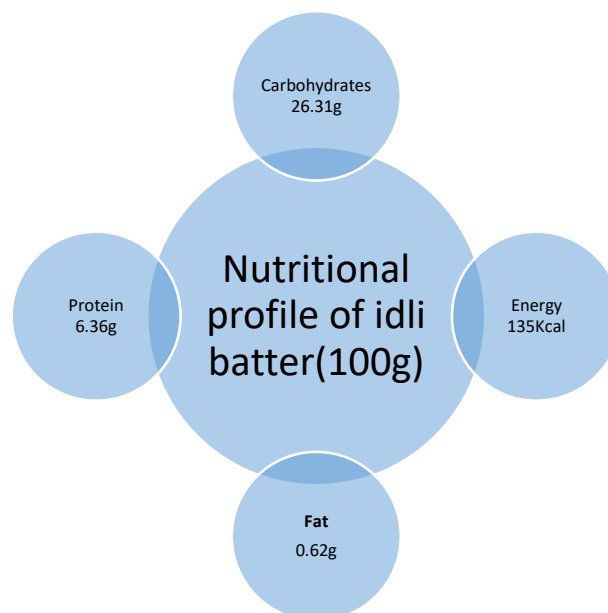


Fig 2.3: Nutritional Composition of Idli (From Kaur *et al.*, 2020)

The exact composition of idli changes based on factors such as the ratio of rice to dal, fermentation time, and the inclusion of additional ingredients like chickpea or soybean flour. Studies have shown that idli prepared with added soy or chickpea flour tends to have higher protein and fiber contents, along with slightly modified fat and carbohydrate levels. The moisture content in idli is generally high due to the steaming process, which makes the final product moist and soft. Protein in idli mainly comes from urad dal, while carbohydrates are primarily contributed by rice. The fat content remains low, making idli suitable for light and healthy diets. These insights are supported by research published in the Journal of Pharmacognosy and Phytochemistry (Kaur *et al.*, 2020) and the International Journal of Current Research and Review (2011).

Parameters	Before fermentation	After fermentation
Bacteria (Log cfu.g ⁻¹)	6.3	10.9
Yeasts (Log cfu.g ⁻¹)	2.6	5.9
pH	6.6	4.9
Volume (ml)	200	410
Amylases (IU.ml ⁻¹)	16.0	22.0
Reducing sugars (mg.g ⁻¹)	3.6	6.0
Free amino acids (mg.g ⁻¹)	8.0	13.0
Crude protein (% w/w)	12.0	12.4
Thiamine (mg.kg ⁻¹)	2.0	7.5
Riboflavin (mg.kg ⁻¹)	2.5	7.5

Fig 2.4: Microbial and physico-chemical changes during idli batter fermentation (from Soni & Sandhu 1991)

2.8 LIMITATION OF NATURAL FERMENTATION

According to Kothari *et al.*, (2019), the major limitations of natural fermentation in idli batter are its dependency on the naturally occurring microbiota present on the raw materials, which leads to variability in fermentation and product quality. The process requires an ambient temperature of 26 to 30°C for microbial metabolism to enable fermentation, and batter prepared from rice alone does not ferment well due to the lack of necessary gas-producing organisms found in urad dal. The natural microbiota is essential for fermentation, as surface sterilized grains show no fermentation, and the fermentation process is relatively slow, typically taking 12 to 15 hours. The study identifies *Weissella* as the major genus responsible for fermentation, emphasizing the complexity and succession of bacterial populations during the process, which impacts acid production, leavening, and overall batter quality.

According to Nisha *et al.*, (2018), the major limitations of natural fermentation in idli batter include the problem of over-fermentation caused by wild indigenous microbes, which leads to puffing and bursting of packets during distribution. Improper storage conditions further exacerbate these issues. High acidity and whey separation during fermentation result in sour-tasting batter and hard-textured cooked idli. Natural fermentation also poses challenges for shelf life, with commonly practiced refrigeration extending it only up to 5-7 days. Thus the microbial growth and acid production, essential for fermentation, can deteriorate batter quality over time, necessitating the use of antimicrobial additives to inhibit excessive microbial activity and extend shelf life without affecting sensory characteristics.

2.9 ROLE OF STARTER CULTURE

According to Kothari *et al.*, (2019), starter cultures play a vital role in idli batter fermentation by enabling a controlled and consistent fermentation process. The study highlights that naturally fermented idli batter involves diverse microbiota, particularly lactic acid bacteria such as *Weissella*, which contribute to acid production and gas formation necessary for leavening. Using starter cultures containing these bacteria improves fermentation efficiency by accelerating acidification and carbon dioxide production, which results in better leavening, texture, aroma, and flavour. It also enhances the nutritional quality and shelf life of the idli batter by suppressing spoilage microbes and reducing fermentation time, thereby standardizing the product quality.

Starter cultures consist of selected strains of lactic acid bacteria (LAB) such as *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Pediococcus cerevisiae*, and *Leuconostoc mesenteroides*, along with yeasts like *Candida versatilis* and *Saccharomyces cerevisiae*. These microbes enzymatically hydrolyze starch and proteins in the batter, resulting in the production of organic acids (mainly lactic acid), carbon dioxide, ethanol, aroma compounds, and bacteriocins. This biochemical activity lowers the pH, improves batter safety by inhibiting spoilage organisms, and enhances the batter's leavening through CO₂ production, which gives idli its characteristic soft, spongy texture.

Momin and Prasad (2019) optimized combinations of lactic acid bacteria and yeast as starter cultures, demonstrating that starter culture inoculation improves batter volume rise, acidity control, and sensory acceptability of the final idli product. Their controlled fermentation approach leads to consistent batter quality compared to natural fermentation, which is subject to variability due to environmental factors and microbial diversity.

2.10 INCORPORATION OF STARTER CULTURES IN CEREAL FERMENTED FOODS

Traditionally, most cereal-based fermented foods across the world rely on natural fermentation or the back-slopping method as their source of microorganisms. However, with evolving trends, these traditional approaches are increasingly being replaced by modern methods—particularly the use of starter cultures as inoculants in fermentation (Brandt, 2014). A starter culture is defined as a microbial preparation containing a high concentration of one or more microorganisms, which is added to raw materials to direct and accelerate the fermentation process (Leroy & de Vuyst, 2004).

Lactic acid bacteria (LAB) and yeasts are the dominant groups of microorganisms used in food fermentations. Advances in modern techniques have enabled the isolation of numerous LAB and yeast strains with specific functional properties. Their roles, however, may vary depending on the fermentation system. For example, *Weissella cibaria*_MG1, an exopolysaccharide (EPS)-producing strain, was studied in sourdough made from different flours (buckwheat, quinoa, oat, teff, and wheat). Results showed that the strain effectively produced EPS in sourdough prepared with buckwheat, quinoa, and teff, but not with oat and wheat (Wolter *et al.*, 2014). This highlights the importance of evaluating starter culture performance directly within the target food system.

Selecting the right strain is a critical step in developing a starter culture. According to (Holzapfel 2002), an ideal starter should enhance product quality by ensuring rapid and consistent metabolic activity, a controlled and predictable fermentation process, desirable sensory attributes, and improved safety through reduced toxicological risks. Additional factors, such as the fermentation substrate and the interactions between strains in mixed cultures, must also be considered. Beyond these, functional traits like probiotic potential, vitamin synthesis, and pathogen inhibition are often desirable.

Today, starter cultures are widely applied in the production of foods such as beer, wine, soy sauce, sauerkraut, and kimchi (Hansen, 2002). In cereal-based foods, their successful use is well established in bread and sourdough production (Coda *et al.*, 2010). However, for many other cereal fermentations, the application of starter cultures remains in the developmental stage, and much of their potential is yet to be explored (Brandt, 2014).

2.11 QUALITY EVALUATION OF IDLI BATTER

According to Momin and Prasad (2019) different changes occurred in quality parameters of idli batter such as pH, acidity and volume of batter. In case of volume there is a rise batter

volume, the idli batter was poured into 100 ml sterilized measuring cylinder, up to 30 ml mark, covered with aluminum foil and was kept at 30 °C for 14 h and observed for the rise in batter volume during fermentation. The increase was measured in ‘ml’ (Sridevi *et al.*, 2010). The pH of the idli batter was determined using a microprocessor-based digital pH meter (pH Tester 30, Elico LI 610, Singapore) as per the procedure described by (Ranganna, 1986). To determine titratable acidity, 10g of fermented batter was taken in a 100ml conical flask to which 20ml of distilled water was added. After adding 3-4 drops of phenolphthalein, the contents were mixed well and titrated against 0.1N NaOH to an endpoint of pale pink colour and expressed as % lactic acid produced (AOAC, 1984).

2.12 QUALITY EVALUATION OF IDLI

Momin and Prasad (2019) analysed the quality of idli mainly by sensory analysis and statistical analysis. In sensory method the Idli prepared from the batter was evaluated based on a 9-points hedonic scale for appearance, flavour, body and texture, colour and appearance and overall acceptability. The sensory evaluation was carried out by an expert panel of 10 trained judges. Whereas in statistical method all experiments were conducted with three replications and the data were subjected to statistical analysis using Completely Randomized Design as per the methods described by Steel and Torrie (1980) and using analysis of variance (ANOVA) by Microsoft Excel Program (Version 7.0). Differences were identified as significant or non-significant based on mean squares and F-test for significance at 5% level of each treatment.

2.13 EVALUATION OF PHYSICO-CHEMICAL PROPERTIES OF BATTER

2.13.1 Measurement of pH

The pH of the idli batter was measured directly using a digital pH meter, following the procedure described by (Kandu *et al.*, 2022).

2.13.2 Titratable acidity assessment

Titratable acidity was determined by blending 10 g of idli batter with 20 mL of distilled water and titrating the mixture against freshly prepared 0.1 N NaOH, with phenolphthalein employed as the indicator. The acidity was expressed as the amount of anhydrous lactic acid produced, according to the method reported by (Dhillon *et al.*, 2020).

2.13.3 Viscosity Measurement

The viscosity of the idli batter (in cP) was determined at 20 rpm using an LV-4 (64) spindle on a Brookfield DV III Rheometer, following the method described by (Kandu *et al.*, 2022).

2.13.4 Percentage Increase in Batter Volume and Whey Separation

Batter samples (25 ml) were transferred to 100 ml measuring cylinders and stored at 30°C for 5 days and at 4°C for 30 days. Batter volume was recorded at five-day intervals, and the percentage increase in volume was calculated as per (Susmitha *et al.*, 2022). Whey separation was evaluated by measuring the whey separated in the same measuring cylinder used for the volume assessment, according to the procedure outlined by (Roy *et al.*, 2023).

2.13.5. Colour Measurement

The colour attributes of the idli batter (L^* , a^* , and b^*) were determined using a KONICA MINOLTA Chroma Meter CR-400, with an 11 mm measurement area, illuminated by a D65 standard light source (pulsed xenon lamp), and data processed using the DP400 system (Karunanithi *et al.*, 2024).

2.14. IDLI BATTER FERMENTATION USING OKARA

Idli fermentation can be accelerated by fortifying the batter with the okara mix. This method has been studied recently. The fortification of soybean in fermented foods is advantageous as increase in digestibility and nutritive value of fermented foods. Natural fermentation of soybean brings desirable changes in taste and texture and may result in the breakdown of some of the anti-nutritional factors. (Deepika *et al.*, 2015). A high protein content of 27–32% (on dry basis) makes okara a potential source of low cost plant origin protein for human nutrition (Chan and Ma, 1999).

2.14.1 PREPARATION OF IDLI BATTER AND FORTIFYING IT WITH OKARA

The 'idli' batter was prepared using three different proportions of rice soji, black gram and okara. To standardize ideal ratio, batters were prepared with different combinations of rice soji, black-gram dhal and okara (3:0.5:0.5, 3:1:1 and 3:1:1.5). This was allowed to ferment for 10 h at room temperature. Total acidity, pH, acidity and the amount of CO released were measured after every 2 hour of natural fermentation time. At the end of natural fermentation, the fermented batter was distributed into hollow depressions (moulds) pans and steam cooked. (Deepika *et al.*, 2015).

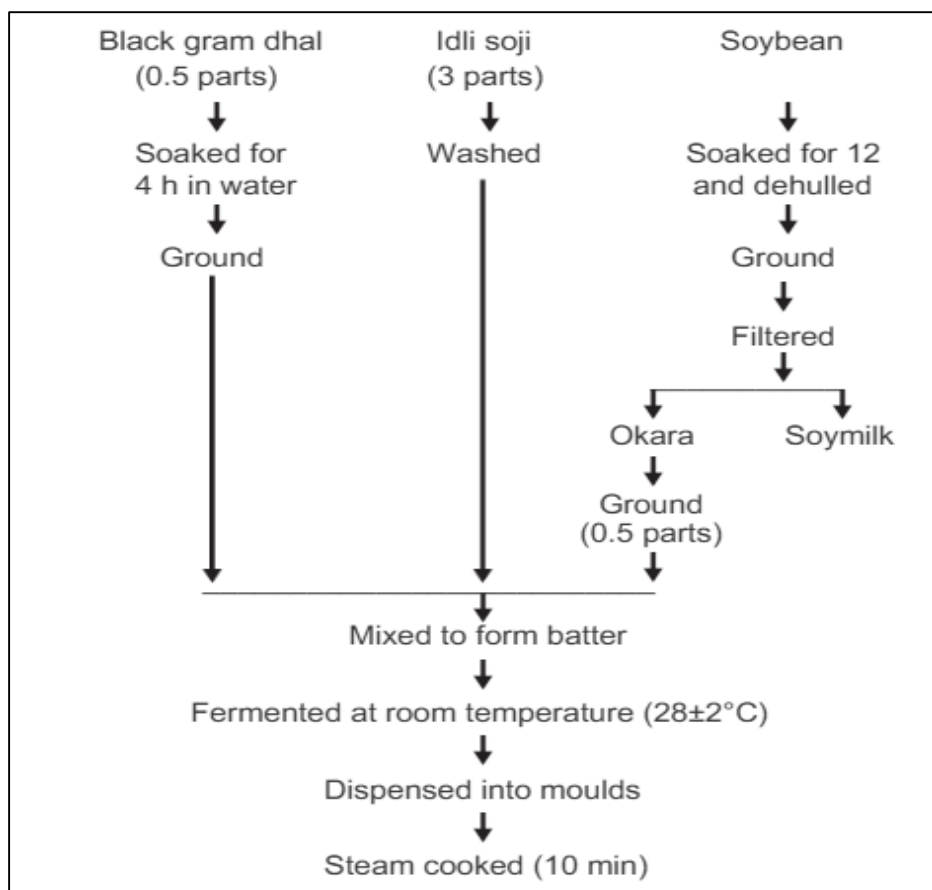


Fig 2.5: Flow chart for idli preparation with okara supplementation (from Deepika et al., 2015)

2.15. ANALYSIS OF THE FORTIFIED BATTER

2.15.1 pH And Titratable Acidity:

The pH of control batter at 0 h was 6.35 which decreased to 4.55 at the end of 14 h of natural fermentation and in okara fortified batter the pH at 0 h was 6.31 and 4.57 at 14 h of natural fermentation. With the progress of natural fermentation, increase in the acid content of batter during natural fermentation was observed in both control and okara fortified 'idli' batter (Rekha *et al.*, 2011). The acidity increased from 0.13 to 0.68 in control batter and from 0.16 to 0.56 in okara fortified batter over a period of 14 h of natural fermentation. The change in pH and acid content can be attributed to the microbial growth especially of lactic acid producing bacteria, which lowers the pH and leavens the batter. The role of LAB is to reduce the pH of the batter to an optimum level (4.4–4.5) for yeast activity. This accounts for fall in pH and rise in acid content of batter as the natural fermentation progresses.

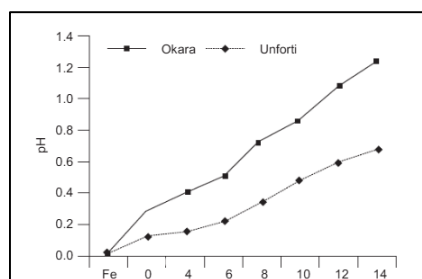


Fig 2.6: Effect of natural fermentation period on pH of control idli batter and okara fortified batter(from Deepika *et al.*, 2015)

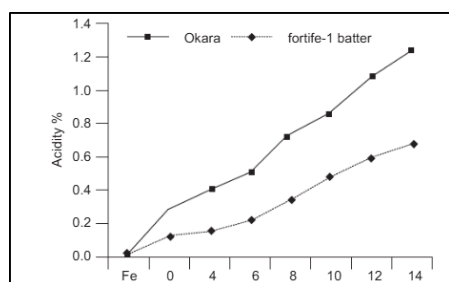


Fig 2.7: Effect of natural fermentation period on total acidity of control idli batter and okara fortified batter(from Deepika *et al.*, 2015)

2.15.2 Batter Volume Analysis:

There was a significant change in the batter volume of the fortified batter. The volume was analysed after keeping the batter for natural fermentation for about 10 hour. The unfortified batter showed a 44% raise and a 70% raise in fortified batter. The fortified batter shown a 26% more raise in volume (Deepika *et al.*, 2015).

2.15.3 CO₂ Analysis:

At 10 h of natural fermentation the percentage of CO₂ released in control was 4.0 and a tremendous increase in CO₂ was seen in okara fortified batter (Deepika *et al.*, 2015). It has also been reported earlier that yeasts are responsible for more than 50% of the CO₂ and two fold increase in the volume of batter (Venkatasubbaiah *et al.*, 1984).

2.16. MICROBIOLOGICAL ANALYSIS OF OKARA FORTIFIED BATTER

The microbial analysis was done on both control and the fortified batter and there was a significant change in the fortified batter. There was an increase in yeast and mold count in the okara fortified batter compared to control batter.

Parameter	Natural Fermentation Period (h)	Type of batter	
		Control	Okara fortified
Lactic count (log10 cfu/g)	0	2.4	3.1
	10	8.2	7.5
Yeast and mold count (log10 cfu/g)	0	1.8	2.7
	10	8.4	9.6
Mesophilic bacteria count (log10 cfu/g)	0	3.2	3.3
	10	8.6	9.2

Fig 2. 8: Microbiological Analysis of Okara Fortified Batter (from Deepika *et al.*, 2015)

It was seen that the bacterial counts were higher in the okara fortified batter than that of control batter (Deepika *et al.*, 2015). This is due to the growth of LAB in sourdough and has been found to be enhanced by the presence of amino acid (Gobetti *et al.*, 1994).

Table 2. Sensory profile of 'idli'				
Parameters	Types of 'idli'			
	S 1	S 2	S 3	S 4
Fluffiness	8.12	5	7.54	8.22
Compactness	7.66	6.23	7.12	7.5
Sponginess	8.28	4.12	7	8.35
Firmness	8.32	5.98	6.75	8.10
Sticky	6.56	6.59	7.52	6.32
Fermented	8.20	7.88	7.96	8.10
Salty	7.58	6	7.18	7
Sour	7.64	6.4	6.99	7.66

*Sample1-3:1(Control), Sample2-3:1:0.5, Sample 3- 3:1:1,
Sample 4- 3:1:1.5*Rice soji: Blackgram: Soy okara

Fig 2.9: Sensory Profile of Okara Fortified Idli (from Deepika *et al.*, 2015)

Table 3. Proximate composition of control 'idli' and okara fortified 'idli'		
Parameters	Control Idli	Okara fortified idli
Moisture (%)	23.51	22.62
Protein (%)	6.12	9.16
Fat (%)	0.54	2.87
Ash (%)	0.23	0.54
Fiber (%)	0.622	2.01
CBH (%)	52.10	42.29

Fig 2.10: Proximate Composition of Control and Fortified Idli (from Deepika *et al.*, 2015)

2.16.1 SENSORY ANALYSIS:

The score of the sensory evaluation is shown in the above table. . For the analysis the control idli was made from the batter which was kept for natural fermentation for 14 hour and the fortified idli was made by the batter which was fortified and kept for the natural fermentation. It has been reported by (Soni and Arora 2000) that yeast involved in the fermentation not only

contribute towards gas production, which results in good texture but also towards the sensory qualities of the 'idli'.

2.16.2. NUTRITIONAL ANALYSIS OF FORTIFIED IDLI BATTER:

It was found that the fortified idli had more nutritional value than the control. As shown in the fig. Due to addition of soy okara the protein content of idli was (9.16%) whereas control idli had (6.12%) protein. Also, the fiber content was more in okara fortified idli (2.01%) and (0.622%) in control idli respectively. The moisture content was nearly same in both idli. Also fat content was more in okara fortified idli (2.87%) than control idli (0.54%). Carbohydrates were significantly more in control idli (52.10%), whereas okara fortified idli had (42.29%) carbohydrates (Deepika *et al.*, 2015).

Thus, from this study, it was found that the fortification of the idli batter with the okara was a beneficial attempt. A higher amount of gas was produced during the fermentation and which induced a good leavening effect on the idli. The increase in CO₂ was found to be 33.5%, which finally resulted in a soft and spongy idli compared to the control unfortified sample. Thus, the fermentation time was brought down to 10 h from the reported 14 h (Deepika *et al.*, 2015) and with improved quality of the final product. Reduction in the fermentation time of the 'idli' batter is of great commercial significance for large scale 'idli' production (Bharti and Laxmi, 2008) and this can be potentially achieved by addition of soy residue okara.

2.17. STARTER CULTURE COMBINATIONS USED IN IDLI FERMENTATION

The use of starter cultures has gained considerable attention as a reliable approach to improve the consistency and quality of idli batter fermentation. Traditional idli fermentation depends on naturally occurring microorganisms present in the raw materials and the surrounding environment. Although this method has been followed for generations, variations in microbial populations and environmental conditions often lead to differences in fermentation time, batter quality, texture, flavour, and shelf life. The introduction of selected starter cultures helps overcome these limitations by ensuring a controlled and reproducible fermentation process (Marco *et al.*, 2021).

Starter cultures used for idli batter generally comprise selected strains of lactic acid bacteria (LAB), yeasts, or a combination of both. Lactic acid bacteria such as *Leuconostoc mesenteroides*, *Lactiplantibacillus plantarum*, *Pediococcus pentosaceus*, and *Lactobacillus fermentum* play a major role in acid production, which lowers the pH of the batter and creates favourable conditions

for fermentation. Yeasts, including *Saccharomyces cerevisiae* and *Candida* species, contribute to the production of carbon dioxide and flavour compounds, resulting in better batter aeration and the characteristic soft, porous texture of steamed idli.

Studies have shown that combinations of LAB and yeast cultures are more effective than individual microorganisms because they work together during fermentation. While LAB produce organic acids that improve microbial stability and flavour, yeasts generate carbon dioxide that increases batter volume and enhances the spongy texture of the final product. This synergistic interaction not only accelerates fermentation but also contributes to better control over the overall fermentation process (Tamang *et al.*, 2020).

Momin and Prasad (2020) investigated the use of different combinations of lactic acid bacteria and yeast as starter cultures for idli batter fermentation. Their findings indicated that starter culture-inoculated batter fermented more rapidly and showed greater batter rise, controlled acidity, and improved sensory quality compared to naturally fermented batter. The use of selected starter cultures also reduced variations between batches, making the fermentation process more consistent and suitable for commercial production.

Recent research has focused on identifying multifunctional starter cultures that not only improve fermentation but also enhance the nutritional and functional properties of fermented foods. Certain LAB strains are capable of producing exopolysaccharides, which improve batter viscosity, moisture retention, and the softness of steamed idli. Some strains also produce antimicrobial compounds that inhibit spoilage microorganisms, thereby improving microbial safety and extending the storage stability of fermented batter (Marco *et al.*, 2021).

The effectiveness of a starter culture largely depends on its ability to grow well in the rice–black gram batter, produce adequate amounts of lactic acid and carbon dioxide, and remain active throughout fermentation and refrigerated storage. Therefore, selecting an appropriate combination of lactic acid bacteria and yeast is an important step in developing a standardized fermentation process that consistently produces idli batter with desirable physico-chemical properties, microbial quality, and sensory characteristics (Leroy and De Vuyst, 2022). Such standardized starter culture combinations have significant potential for improving the quality, consistency, and commercial production of ready-to-cook idli batter.

2.18 OPTIMIZATION OF FERMENTATION PARAMETERS

Optimization of fermentation parameters is essential for developing a standardized idli batter with consistent quality and improved processing efficiency. Since fermentation is influenced by several interacting factors, identifying the optimum conditions is necessary to achieve desirable physico-chemical characteristics, microbial stability, and sensory quality. Parameters such as starter culture concentration, fermentation time, incubation temperature, batter consistency, and rice-to-black gram ratio significantly influence the fermentation process and require careful optimization.

Among these variables, the concentration of starter cultures plays an important role in determining the rate of fermentation. An appropriate inoculum level promotes rapid establishment of beneficial microorganisms and ensures controlled fermentation, whereas very low inoculum levels delay fermentation and excessive inoculation may lead to rapid acid development and undesirable flavour changes. Therefore, selecting the optimum concentration of starter cultures is important for obtaining uniform fermentation and consistent product quality.

Fermentation time is another important parameter that requires optimization. Studies have shown that the use of defined starter cultures can considerably reduce fermentation time compared with spontaneous fermentation while maintaining desirable batter characteristics. Controlled fermentation not only improves process efficiency but also minimizes variations associated with seasonal and environmental changes (Momin and Prasad, 2020).

The composition of the batter also influences fermentation efficiency. The rice-to-black gram ratio determines the availability of carbohydrates and proteins required for microbial growth, while the water content affects batter consistency and microbial activity. Maintaining suitable formulation ratios facilitates uniform fermentation and improves the reproducibility of the process. Optimization of these formulation variables is therefore essential for obtaining batter with consistent quality during commercial production.

Recent studies have increasingly adopted statistical optimization techniques to evaluate the combined effects of multiple process variables. Experimental design methods enable simultaneous assessment of fermentation parameters and their interactions, reducing the number of experimental trials while providing reliable prediction of optimum processing conditions. Such approaches improve process standardization, minimize experimental cost, and enhance reproducibility, making them highly suitable for industrial applications. The optimization of fermentation

parameters is particularly important for commercial production of ready-to-cook idli batter, where consistency between production batches is essential. A standardized fermentation process ensures predictable microbial activity, uniform product quality, and improved storage stability. Consequently, optimization of starter culture concentration and fermentation conditions forms the basis for developing a reliable and commercially viable fermentation process.

2.19. SHELF-LIFE STUDIES OF IDLI BATTER

Shelf life is one of the major challenges in the commercial production of ready-to-cook idli batter. Even under refrigerated conditions, continuous microbial activity during storage leads to gradual acidification, whey separation, changes in batter consistency, and deterioration of sensory quality. Consequently, extending the shelf life of fermented idli batter while preserving its desirable characteristics has become an important area of research. Deepika *et al.*, (2015) reported that fortification of idli batter with soy okara accelerated fermentation and improved batter quality by enhancing carbon dioxide production and batter rise. The fortified batter produced softer and more acceptable idli while reducing the fermentation time from 14 h to 10 h. The study suggested that maintaining appropriate storage conditions is necessary to preserve the improved quality of the fermented batter during storage.

Anitha *et al.*, (2016) investigated the incorporation of curry leaf powder as a natural functional ingredient in idli batter. In addition to improving the nutritional quality of the product, curry leaves exhibited antimicrobial activity that delayed microbial spoilage and extended the shelf life of batter stored at ambient temperature from approximately two days to five days without adversely affecting the sensory properties of the final product.

Nisha *et al.*, (2018) evaluated mustard essential oil as a natural preservative for fermented idli batter. The incorporation of mustard essential oil effectively reduced microbial growth, delayed acidification, and improved storage stability without significantly affecting the texture, colour, or overall acceptability of steamed idli. The authors concluded that natural preservatives could effectively improve the keeping quality of fermented batter while maintaining consumer acceptability.

Varadaraj *et al.*, (2014) demonstrated that batter prepared using defined starter cultures exhibited better storage stability than naturally fermented batter. During refrigerated storage, starter culture-fermented batter showed lower deterioration in texture and sensory quality while maintaining acceptable acidity. The authors reported that controlled fermentation using selected

combinations of *Enterococcus faecium*, *Pediococcus pentosaceus*, and *Candida versatilis* improved the storage quality of batter compared with spontaneous fermentation. More recently, Karnat *et al.*, (2024) investigated the changes in the functional properties of fermented idli batter during storage under ambient (30°C) and refrigerated (5°C) conditions. The study monitored physico-chemical, microbiological, and textural changes and reported that refrigeration significantly slowed quality deterioration by maintaining batter consistency and delaying the increase in acidity. Refrigerated samples remained acceptable for up to nine days, whereas batter stored at ambient temperature deteriorated within 48 hours. In a related study, Karunanithi *et al.*, (2024) developed a betel leaf essential oil-based nano-emulsion as a natural preservative for fermented idli batter. The optimized nano-emulsion effectively controlled microbial growth and maintained pH, titratable acidity, viscosity, colour, and sensory attributes during storage. The treatment extended the shelf life of idli batter to seven days under ambient conditions and up to twenty-five days under refrigerated storage (4°C).

Collectively, these studies demonstrate that the shelf life of idli batter can be effectively enhanced through the use of selected starter cultures, natural antimicrobial agents, and appropriate refrigerated storage. These approaches help maintain the physico-chemical, microbiological, and sensory quality of the batter, thereby improving its commercial potential and consumer acceptability.

2.20. DESIGN-EXPERT® SOFTWARE

Design-Expert® software is a widely used statistical package for designing experiments, analysing data, and optimizing process variables in food research. The software integrates Design of Experiments (DOE) with Response Surface Methodology (RSM), allowing researchers to evaluate the individual and interactive effects of multiple independent variables on one or more responses. It supports various experimental designs, including Central Composite Design (CCD), Box–Behnken Design (BBD), factorial, and mixture designs, making it a versatile tool for process optimization (Anderson and Whitcomb, 2017).

Several researchers have employed Design-Expert® software in food processing studies to optimize extraction processes, fermentation conditions, drying operations, formulation development, and preservation techniques. Md. Yusof *et al.*, (2019) used the software to optimize ultrasound-assisted extraction of flavonoids from cocoa bean shell, while Karunanithi *et al.*, (2024) optimized a betel leaf essential oil-based nano-emulsion for extending the shelf life of fermented

idli batter. The optimized formulation effectively maintained the physico-chemical, microbiological, and sensory quality of the batter during storage. Although only a few studies have directly applied Design-Expert® software for optimizing idli batter fermentation, it has been widely adopted in fermentation and food process optimization because of its ability to evaluate interactions among multiple variables, develop predictive mathematical models, and identify optimum processing conditions with fewer experimental trials. Consequently, Design-Expert® software has become a valuable tool for process standardization and product development in food science and technology.

2.21. RATIONALE AND RELEVANCE OF THE WORK

Idli is one of the most popular traditional fermented foods in India and is widely appreciated for its nutritional value, easy digestibility, and desirable sensory characteristics. Despite its widespread consumption, the quality of fermented idli batter is largely influenced by the naturally occurring microflora and environmental conditions during fermentation. Such variations often result in inconsistent fermentation, leading to differences in batter quality, texture, flavour, and storage stability.

The literature reviewed in this chapter indicates that the use of selected starter cultures offers significant potential for improving the consistency and efficiency of idli batter fermentation. Controlled fermentation using suitable combinations of lactic acid bacteria and yeast has been reported to enhance batter leavening, improve physico-chemical and sensory properties, and minimize batch-to-batch variations. In addition, optimization of fermentation parameters and the application of statistical tools have been shown to contribute to better process control and product standardization. Recent studies have also highlighted the importance of improving the shelf life of ready-to-cook idli batter through controlled fermentation, refrigeration, and the use of natural preservatives.

With the growing consumer preference for convenient and ready-to-cook fermented foods, there is an increasing demand for standardized processing techniques that ensure consistent quality, safety, and extended storage stability. Therefore, standardization of the fermentation process using selected starter culture combinations has considerable scientific and industrial relevance. Such an approach is expected to improve process reproducibility, enhance product quality, and support the development of commercially viable idli batter with greater consumer acceptance.

2.22 RESEARCH GAP

Idli batter fermentation has been widely studied over the years because of its importance in improving the nutritional quality, texture, flavour, and keeping quality of the final product. Early research mainly focused on understanding the microorganisms involved in natural fermentation and their role in the development of desirable physico-chemical and sensory characteristics. More recent studies have explored the use of selected starter cultures, functional ingredients, natural preservatives, and improved storage methods to produce batter with better quality and extended shelf life. These findings have provided valuable insights into the fermentation process and have demonstrated the advantages of controlled fermentation over the conventional spontaneous method.

However, despite the progress made, certain aspects of idli batter fermentation remain insufficiently explored. Most studies have evaluated the effect of individual starter cultures or specific processing conditions, while only limited attention has been given to the use of combinations of selected lactic acid bacteria and yeast cultures. In addition, the combined influence of important fermentation variables such as starter culture concentration, fermentation time, and incubation conditions on the physico-chemical, microbiological, and sensory quality of idli batter has not been studied comprehensively. As a result, the development of a standardized fermentation process capable of producing consistent batter quality remains a challenge.

In recent years, statistical optimization techniques such as Response Surface Methodology (RSM) and Design-Expert® software have been widely used in food process optimization to evaluate the interaction between multiple processing variables and to identify optimum operating conditions. Although these approaches have been successfully applied in several areas of food processing, their application in the optimization of idli batter fermentation using selected starter culture combinations is still limited. A systematic evaluation of these factors is therefore essential to better understand their interactions and their effect on fermentation efficiency, batter quality, sensory attributes, and storage stability.

Bridging these research gaps would contribute to the development of a scientifically standardized fermentation process for idli batter, ensuring consistent product quality and improved shelf life. Such an approach would also provide useful information for the commercial production of ready-to-cook idli batter and support the adoption of optimized fermentation techniques in the food industry.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

This chapter presents a detailed investigation into the standardization of idli batter using selected lactic acid bacterial starter cultures. The raw materials used, methods of preparation, physico-chemical and microbiological analyses, experimental design, and shelf-life evaluation of the fermented idli batter are described in detail in the following sections.

3.1 COLLECTION OF RAW MATERIALS

The primary raw materials used for the preparation of idli batter were parboiled rice (*Oryza sativa*) and de-hulled black gram, commonly known as urad dal (*Vigna mungo*). Both ingredients were procured from a reputed local market and carefully selected for their uniform grain size, absence of visible defects, foreign matter, and insect damage. The quality of the raw materials is a critical determinant of the batter characteristics, fermentation behaviour, and final textural quality of the idli.

Starter cultures selected for this study were *Lactococcus lactis*, *Lactobacillus plantarum*, and *Lactobacillus fermentum*. These cultures were purchased as skim milk culture from the Department of Dairy Microbiology, Verghese Kurien Institute of Dairy and Food Technology (VKIDFT). The purchased cultures were sub cultured to nutrient broth to maintain their viability and activity. For long-term storage, the actively growing culture was centrifuged to obtain a concentrated cell pellet, which was retained in this concentrated form. *Lactococcus lactis* is a homofermentative lactic acid bacterium widely used in dairy and fermented food applications for its rapid acid production and flavour development capabilities. *Lactobacillus plantarum* is a heterofermentative species known for its robustness, broad substrate range, and production of bacteriocins that suppress spoilage organisms. *Lactobacillus fermentum* contributes to the aroma profile of fermented products through the production of acetic acid, ethanol, and carbon dioxide in addition to lactic acid. The concentrated cell pellets of all three starter cultures were stored under refrigerated conditions at 4 ± 1 °C until further use to preserve their viability, metabolic activity, and fermentative potential.

3.2 PREPARATION OF IDLI BATTER

Idli batter is a naturally fermented mixture of ground rice and urad dal that forms the substrate for steamed idli, one of the most widely consumed traditional South Indian foods. The preparation of a well-balanced batter requires careful attention to the ratio of ingredients, soaking duration, grinding technique, and fermentation conditions, all of which collectively influence the physical, chemical, and sensory characteristics of the final product.

Parboiled rice and de-hulled urad dal were weighed in a 2:1 ratio (w/w) as per the standard practice for idli batter preparation. Both ingredients were cleaned manually to remove dirt, stones, and other extraneous matter, and washed thoroughly under running water until the washings ran clear. The rice and dal were soaked separately in sufficient clean water for 4-6 hours at room temperature (28–30 °C) to facilitate complete hydration of the starch granules and protein bodies. Adequate soaking ensures softer grinding, better starch swelling, and improved fermentation by increasing the water activity of the substrate.

After soaking, the excess water was drained from both the fractions. Urad dal was ground first in a laboratory grinder with a minimal quantity of chilled water to obtain a smooth, light, and highly aerated batter. The aeration at this stage is crucial as it incorporates air bubbles into the protein matrix of the dal, contributing to batter expansion during fermentation. The soaked rice was subsequently ground to a slightly coarse consistency, which helps retain a desirable granular texture in the steamed idli. The two ground fractions were combined in a clean vessel and mixed uniformly, with water added in appropriate quantities to achieve a freely pourable consistency with a total solids content suitable for fermentation and steaming.

For the inoculated batches, starter cultures were prepared and added differently depending on the stage of the study. During the trial-and-error experiments, the organisms were inoculated directly from the skim milk culture into the base batter. For the shelf-life study, a more precise inoculum preparation method was followed: each starter culture was first grown individually in sterile nutrient broth at 37°C for 18–24 hours. The grown culture was then centrifuged at 5000 rpm for 15 minutes to obtain a concentrated cell pellet. The pellet was washed 3 times with sterile distilled water and finally diluted with the same to prepare the working inoculum suspension, which was then added to the idli batter at concentrations of 0.2% (v/w), 0.3% (v/w), and 0.5% (v/w). Each 100 g batch of base batter received the designated volume of inoculum (0.2 mL, 0.3

mL, or 0.5 mL per 100 g batter, respectively) and was mixed gently using a sterile spatula to ensure uniform distribution throughout the batter matrix. The inoculated batter was fermented in a controlled temperature incubator maintained at 30–35°C for designated time periods of 2, 4, and 6 hours under hygienic conditions. Following fermentation, the batter from each batch was evaluated for physico-chemical parameters including pH, titratable acidity, colour (L^* , a^* , b^*), bulk density, and volume rise as described in Section 3.3.1. Microbiological quality was assessed by total plate count as described in Section 3.3.2. Fresh idlis were prepared from each batch by steaming the fermented batter in idli moulds for approximately 10–12 minutes and subjected to sensory evaluation using a 9-point hedonic scale.

3.3 DETERMINATION OF PHYSICO-CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF IDLI BATTER

Prior to and following standardization, the physico-chemical and microbiological properties of idli batter were systematically evaluated to characterize the quality changes induced by different starter culture combinations, inoculum concentrations, and fermentation durations. The parameters selected reflect the fermentation activity, acid profile, physical structure, and microbial safety of the batter. The methods employed for each parameter are described in detail under the following subsections.

3.3.1 Physico-Chemical Properties of Idli Batter

3.3.1.1 Colour

Colour is an important quality attribute of idli batter and the resulting idli, as it is one of the first characteristics perceived by the consumer and directly influences purchase intent and overall acceptability. The colour of idli batter is affected by the raw material composition, degree of milling, soaking conditions, fermentation extent, and heat treatment during steaming. Colour measurement provides an objective, reproducible, and instrument-based evaluation in contrast to subjective visual assessment.

The colour characteristics of the idli batter were evaluated using a Lovibond colourimeter (The Tintometer Ltd., Salisbury, UK), as shown in Plate 3.1. The instrument operates on the principle of measuring the intensity of light reflected from the surface of the sample across the entire visible spectrum. The standard observer curves of the colourimeter are based on red, green,

and blue tristimulus values, which are then mathematically converted to the CIE L^* , a^* , b^* colour space.

In the CIE L^* , a^* , b^* system, L^* represents the lightness coordinate, ranging from 0 (absolute black) to 100 (absolute white), and reflects the brightness of the sample. The a^* value describes the chromatic component along the green (negative a^*) to red (positive a^*) axis, while the b^* value indicates the chromatic component along the blue (negative b^*) to yellow (positive b^*) axis (Reddy *et al.*, 2014). An increase in b^* value during fermentation may indicate yellowing due to enzymatic browning or Maillard-type reactions, while changes in L^* reflect the degree of whitening or darkening of the batter matrix.

Before commencing sample analysis, the colourimeter was calibrated using manufacturer-provided black and white reference tiles to zero the instrument and standardize the reflectance values. The idli batter sample was placed in a clean, transparent glass sample cup ensuring a uniform, bubble-free surface, and the cup was carefully positioned over the measurement port. The colour values L^* , a^* , and b^* were recorded directly from the digital display. All measurements were performed in triplicate at room temperature and the mean values were used for analysis.



Plate 3.1 Lovibond Tintometer

3.3.1.2 Titratable Acidity

Titrateable acidity (TA) is a fundamental quality parameter in fermented foods that quantifies the total acid content of a sample by determining the amount of alkali required to neutralize all acidic species present. In the context of idli batter fermentation, titrateable acidity

reflects the cumulative concentration of organic acids, primarily lactic acid, produced through the metabolic activity of lactic acid bacteria. Unlike pH, which measures only the concentration of free hydrogen ions in solution, titratable acidity accounts for both dissociated and undissociated acid fractions and therefore provides a more comprehensive measure of the total acid load in the batter.

During fermentation of idli batter, heterofermentative and homofermentative lactic acid bacteria ferment glucose, maltose, and other fermentable sugars present in rice and urad dal to produce lactic acid as the predominant metabolic end product. The progressive increase in titratable acidity over fermentation time and during refrigerated storage is a direct indicator of continued microbial activity and acid accumulation. Monitoring TA at different storage intervals allows assessment of the rate of acidification, the shelf stability of the batter, and the threshold beyond which excessive acidity leads to sensory deterioration of the idli.

Titration acidity was expressed as percentage lactic acid, since lactic acid is the principal organic acid produced during idli batter fermentation. The selected batter sample was analysed for TA at storage intervals of 0th, 7th, and 14th day. For determination, approximately 10 g of idli batter was accurately weighed using an analytical balance and transferred quantitatively to a clean 250 mL conical flask. To this, 90 mL of distilled water was added, and the mixture was thoroughly homogenized by stirring. Two to three drops of phenolphthalein indicator (1% w/v in 95% ethanol) were added to the suspension. The sample was titrated against standardized 0.1 N sodium hydroxide (NaOH) solution taken in a burette, with continuous swirling, until the appearance of a faint but persistent pink colour that lasted for at least 30 seconds indicated the neutralization endpoint. All analyses were performed in triplicate and the mean values were reported. The titration acidity was calculated using the following formula:

$$\text{Percentage acidity of idli batter} = \frac{10 \times (\text{ml of } 0.1 \text{ N NaOH}) \times 0.009008 \times 100}{\text{weight of the sample (g)}}$$

Equation 3.1 Titratable Acidity

3.3.1.3 pH Measurement

The pH of a food system is defined as the negative logarithm of the hydrogen ion concentration and is a primary indicator of the acidity or alkalinity of the medium. In fermented

products such as idli batter, pH is a critical parameter that reflects the instantaneous acid concentration resulting from microbial metabolic activity and is intimately linked to the growth kinetics of fermenting organisms, the inhibition of pathogenic and spoilage bacteria, the rate of starch hydrolysis, and the texture and flavour development of the final product. A decrease in pH during fermentation is directly attributable to the production of lactic acid and other organic acids by lactic acid bacteria, which lower the hydrogen ion concentration in the batter.

The rate and extent of pH drop during fermentation depends on several factors including the initial microbial load, substrate composition, buffering capacity of the batter, temperature, and the types and concentrations of starter cultures inoculated. Excessively low pH values indicate over-fermentation and result in a highly sour product with compromised consumer acceptability. pH monitoring at successive storage intervals therefore provides a reliable index of fermentation progress and batter quality over time.

The pH of idli batter was measured using a digital pH meter as shown in Plate 3.2. The pH meter works on the principle of the potentiometric measurement of hydrogen ion activity using a glass electrode in conjunction with a reference electrode. The potential difference generated between the glass electrode and the reference electrode in the sample is directly proportional to the pH of the solution. Before each set of measurements, the pH meter was calibrated using standard buffer solutions of pH 4.0, 7.0, and 9.0 in sequence to ensure linearity and accuracy of the readings across the expected measurement range. The electrode was rinsed thoroughly with distilled water and blotted dry between readings to avoid cross-contamination.

A representative portion of freshly prepared or refrigerated idli batter was transferred to a clean and dry beaker. The electrode was fully immersed in the batter, ensuring that the sensing bulb did not touch the sides or bottom of the container, and the batter was gently agitated to ensure homogeneity. The reading was allowed to stabilize for at least one minute before recording. Each sample was measured at room temperature, and the average value was noted. pH readings were taken at the 0th, 7th, 14th, and 21st day of refrigerated storage to track the acidification pattern during shelf life.



Plate 3.2. pH Meter

3.3.1.4 Bulk Density of Idli Batter

Bulk density is defined as the mass of a material per unit volume under a specified set of conditions. In the context of idli batter, bulk density is a key physical parameter that provides a direct and quantitative measure of the degree of aeration within the batter matrix. It serves as an indirect index of fermentation efficiency, as the entrapment of carbon dioxide gas produced by lactic acid bacteria and yeasts during fermentation causes the batter to expand, thereby occupying a larger volume for the same mass, and consequently reducing bulk density.

The relationship between fermentation activity and bulk density is well established in the literature on leavened and fermented batters. At the initiation of fermentation, the batter has a compact, dense structure with minimal entrapped gas, resulting in a relatively high bulk density. As fermentation progresses, the metabolic activity of fermenting microorganisms intensifies, leading to increased gas production, formation of gas bubbles, and progressive expansion of the batter volume. The development of a stable protein-starch gel network in urad dal batter plays an important role in retaining these gas bubbles, which are essential for the characteristic soft and spongy texture of idli.

A reduction in bulk density with increasing fermentation time reflects optimal microbial activity and batter expansion, and correlates positively with the lightness, softness, and sponginess of the steamed idli. Conversely, an excessively low bulk density may indicate over-fermentation resulting in the collapse of the gas bubble structure, leading to a poorly textured final product.

In the present study, bulk density was measured before fermentation and at 2-hour, 4-hour, and 6-hour intervals following inoculation. A clean, dry graduated cylinder of 20 mL capacity was used. The idli batter sample was gently poured into the cylinder up to the 20 mL mark without

tapping, shaking, or compressing, so as to avoid the collapse of air cells entrapped in the batter matrix. The total weight of the cylinder with the batter was measured using a calibrated digital analytical balance, and the weight of the empty cylinder was subtracted to obtain the net weight of the batter. Bulk density was calculated using the formula:

$$\text{Bulk Density (g/mL)} = \frac{\text{Weight of batter (g)}}{\text{Volume of batter (mL)}}$$

Equation 3.2 Bulk Density

3.3.1.5 Volume Rise of Idli Batter

Volume rise is a direct and visually evident physical parameter used to quantify the extent of gas production and retention within the idli batter during fermentation. It is one of the most practical indicators of fermentation activity and batter quality, as it reflects the cumulative effect of microbial gas production, the structural integrity of the batter network, and the ability of the batter matrix to entrap and retain the generated gas without collapse.

During fermentation, the carbohydrates present in rice and urad dal are metabolized by lactic acid bacteria and associated fermentative organisms to produce carbon dioxide as a metabolic by-product. Carbon dioxide is produced primarily through heterofermentative pathways and through the decarboxylation reactions carried out by certain *Lactobacillus* species. As fermentation proceeds, these gas molecules are trapped within the viscoelastic gel network formed by the proteins and gelatinized starch of urad dal, leading to progressive inflation of the batter and a measurable increase in its volume.

The volume rise of idli batter is influenced by multiple factors including the composition of the starter culture, the concentration of the inoculum, the fermentation temperature, the duration of fermentation, the rice-to-dal ratio, water content, and the buffering capacity of the batter. A higher volume rise generally correlates with greater fermentation activity, better batter leavening, and improved softness and porosity of the steamed idli. However, excessive or prolonged fermentation may cause the batter network to weaken, resulting in gas escape, volume collapse, and undesirable sourness in the final product.

Volume rise was measured as the increase in batter height or volume from the initial level. At each designated time interval, the batter volume in a graduated container was noted and the percentage increase over the initial volume was calculated and recorded. Measurements were taken at 0, 2, 4, and 6 hours of fermentation, and additionally at the 0th, 7th, and 14th day of refrigerated storage to monitor changes in the fermentation potential of the batter over time.

3.3.2 Microbiological Analysis of Idli Batter

3.3.2.1 Total Plate Count

Microbiological quality of fermented food products is a critical safety and quality parameter that determines the acceptability of the product for human consumption. The total plate count (TPC), also referred to as the aerobic mesophilic count, estimates the total number of viable, culturable bacteria present in a food sample under specified incubation conditions. In the context of idli batter, TPC provides information on both the fermentation-associated microbial load and the presence of any incidental spoilage or hygiene-indicator organisms. It is a widely accepted index for assessing the overall microbiological quality and safety of fermented batters during processing and storage.

An elevated TPC in freshly fermented idli batter is expected, as it reflects the high population of lactic acid bacteria and other fermenting microorganisms responsible for the desired physico-chemical transformations. However, during refrigerated storage, an increase in TPC beyond permissible limits indicates microbial proliferation due to psychrotrophic organisms, which can lead to quality deterioration, off-flavour development, excessive acidification, and potential public health risk. Monitoring TPC at regular storage intervals is therefore essential to establish the shelf life and safety boundaries of the standardized idli batter.

The total plate count of the idli batter was determined using the standard pour plate technique. The pour plate method is based on the principle that each viable bacterial cell present in the diluted sample, when uniformly distributed in a suitable solid agar medium, will germinate and develop into a visible, discrete, and countable colony during incubation. The number of colonies per plate, multiplied by the dilution factor, gives the total viable count per gram or millilitre of the original sample.

Five grams of fermented idli batter were aseptically weighed using a sterile spatula and transferred into a sterile flask containing 10 mL of sterile distilled water, yielding an initial 1:2 dilution. The contents were shaken vigorously to achieve uniform dispersion of microbial cells. Serial decimal dilutions were prepared by successively transferring 1 mL of the diluted suspension into test tubes containing 9 mL of sterile distilled water (with thorough mixing at each step). The dilution series was extended up to 10^{-6} to ensure that appropriate countable dilutions were obtained from samples with varying microbial loads. All dilution steps were performed under aseptic conditions using sterile pipettes to avoid contamination.

One millilitre aliquot from selected dilutions were transferred aseptically into sterile, labelled Petri plates in duplicate. Molten Plate Count Agar (PCA), previously sterilized by autoclaving at 121°C for 15 minutes and tempered, was poured into each plate to a depth of approximately 4 mm. The plates were gently rotated in a fig-of-eight motion to ensure uniform mixing of the sample with the medium, and the agar was allowed to solidify at room temperature. After solidification, the plates were inverted to prevent condensation of water from dripping onto the colonies and incubated at 37°C for 24–48 hours. After the incubation period, plates showing between 30 - 300 well-separated colonies were selected, and the total number of colonies was counted using a digital colony counter. The TPC was calculated using the equation:

$$\text{CFU} = \frac{(\text{Number of colonies} \times \text{dilution factor})}{\text{Volume plated}}$$

Equation 3.3 Bulk Density

3.4 EXPERIMENTAL DESIGN FOR STANDARDIZATION OF IDLI BATTER

The experimental design for the standardization of idli batter using lactic acid bacterial starter cultures was formulated to systematically evaluate the influence of three independent variables, namely the type of starter culture combination, the concentration of inoculum (%), and the duration of fermentation (hours), on the physico-chemical quality and sensory acceptability of idli batter. The primary objective of the experimental design was to identify the optimal combination of these variables that consistently yields idli batter with the best fermentation activity, desirable physico-chemical properties, and the highest sensory scores for the resulting idli.

A total of 17 trial combinations were designed using a software-based experimental planning tool to ensure systematic coverage of the variable space while minimizing experimental effort. The independent variables and their levels were selected based on a review of the existing literature on idli fermentation and preliminary exploratory experiments. The response variables measured for each trial included volume rise, bulk density, pH, titratable acidity, total plate count, and sensory evaluation scores.

3.4.1 Preparation of Idli Batter for Experimental Trials

A standardized batch of idli batter was prepared as described in Section 3.2 prior to each set of experimental trials. Parboiled rice and de-hulled urad dal were weighed in a 2:1 ratio, cleaned, soaked separately for 4-6 hours, and ground to the appropriate consistency using a laboratory grinder. The ground fractions were mixed to obtain a uniform base batter. Portions of 100 g of the base batter were weighed accurately using a digital balance and transferred into 500 g low-density polyethylene (LDPE) pouches. The starter culture inoculums for the trial-and-error experiments were prepared directly from the skim milk culture maintained at the Department of Dairy Microbiology, VKIDFT. The requisite volume of each skim milk culture was measured and added directly to the batter at the specified concentrations of 0.2% (v/w), 0.3% (v/w), and 0.5% (v/w) per 100 g of base batter, corresponding to 0.2 mL, 0.3 mL, and 0.5 mL of inoculum per batch, respectively. The inoculums were added to the batter in the pouches, mixed gently to ensure homogeneous distribution, and the pouches were sealed. Details of the starter culture combinations, inoculum concentrations, and batch allocations used in the trial combinations are summarized in Table 3.2

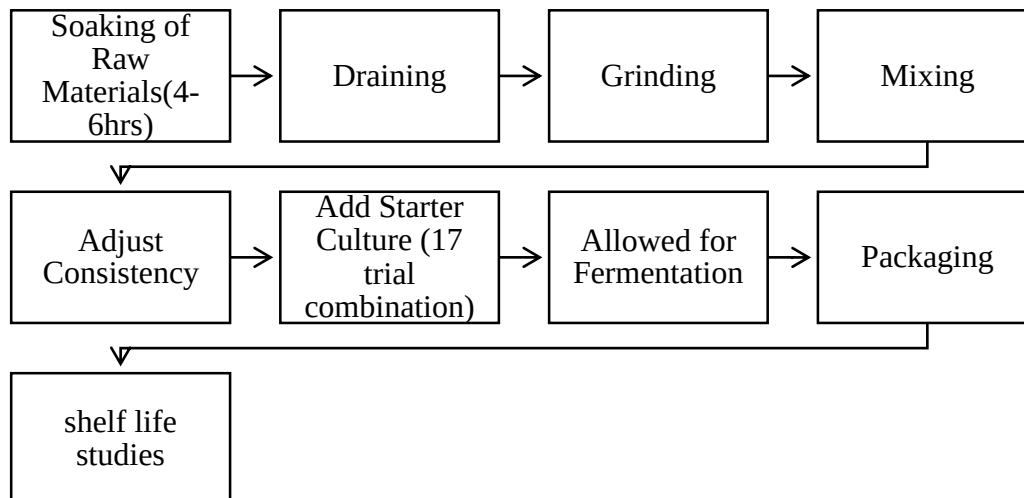


Fig 3.1 Process Flowchart for the Preparation of Idli Batter

3.4.2 Trial Combinations Using Experimental Design

The experiment was designed using three independent variables, namely type of culture, Time, and Concentration. These process variables were selected based on their significant influence on the performance and quality characteristics of the developed system/product. Response Surface Methodology (RSM) was employed to optimize the selected process variables and to evaluate the interactive effects among them. A Box–Behnken experimental design, comprising independent variables as described was adopted for the optimization study. The design was selected because it provides an efficient experimental framework for developing second-order polynomial models while requiring fewer experimental runs than other response surface designs.

The experimental responses obtained from each trial were analysed using appropriate statistical tools to establish mathematical relationships between the independent variables and the response variables. The developed regression models were used to predict the optimum combination of process parameters that would result in the desired product quality and process performance.

The optimization of the process parameters was carried out based on the following dependent variables Colour, Titratable acidity, pH, Density, and Sensory evaluation. The experimental data generated from the Box–Behnken design were subjected to analysis of variance (ANOVA) to determine the significance of the developed models and the individual process variables. The adequacy of the regression models was evaluated using statistical parameters such

as the coefficient of determination (R^2), adjusted R^2 , predicted R^2 , lack-of-fit test, and the significance (p-value) of the model terms. The optimum process conditions were identified by simultaneously optimizing the selected response variables using the desirability function approach provided in the Response Surface Methodology. The optimized conditions were subsequently validated experimentally to verify the accuracy and reliability of the predicted results.

Table 3.1 Experimental Design for Standardization of Idli Batter Using Starter Cultures

Std	Run	Factor 1 A:Culture No	Factor 2 B:Time (hour)	Factor 3 C:Concentration (mg)
13	1	2	5	0.2
17	2	2	5	0.2
2	3	3	4	0.2
14	4	2	5	0.2
7	5	1	5	0.3
16	6	2	5	0.2
11	7	2	4	0.3
6	8	3	5	0.1
15	9	2	5	0.2
9	10	2	4	0.1
4	11	3	6	0.2
5	12	1	5	0.1
10	13	2	6	0.1
1	14	1	4	0.2
12	15	2	6	0.3
3	16	1	6	0.2
8	17	3	5	0.3

Table 3.2: Trial Combinations Used in the Experimental Design for Standardization of Idli Batter

Run	Code	Starter Culture Combination	Concentration (%, v/w)	Fermentation Time (h)
1	Control	No starter culture (natural microflora)	–	–
2	LY	<i>Lactococcus lactis</i> + <i>Saccharomyces cerevisiae</i>	0.1	2
3	LPF	<i>Lactococcus lactis</i> + <i>Lactobacillus plantarum</i> + <i>Lactobacillus fermentum</i>	0.2	2
4	LP	<i>Lactococcus lactis</i> + <i>Lactobacillus plantarum</i>	0.2	2
5	LY	<i>Lactococcus lactis</i> + <i>Saccharomyces cerevisiae</i>	0.3	2
6	LP	<i>Lactococcus lactis</i> + <i>Lactobacillus plantarum</i>	0.3	4
7	LPF	<i>Lactococcus lactis</i> + <i>Lactobacillus plantarum</i> + <i>Lactobacillus fermentum</i>	0.1	4
8	LPF	<i>Lactococcus lactis</i> + <i>Lactobacillus plantarum</i> + <i>Lactobacillus fermentum</i>	0.3	4
9	LY	<i>Lactococcus lactis</i> + <i>Saccharomyces cerevisiae</i>	0.2	4
10	LP	<i>Lactococcus lactis</i> + <i>Lactobacillus plantarum</i>	0.1	4
11	LPF	<i>Lactococcus lactis</i> + <i>Lactobacillus plantarum</i> + <i>Lactobacillus fermentum</i>	0.3	6
12	LY	<i>Lactococcus lactis</i> + <i>Saccharomyces cerevisiae</i>	0.3	6
13	LP	<i>Lactococcus lactis</i> + <i>Lactobacillus plantarum</i>	0.2	6
14	LY	<i>Lactococcus lactis</i> + <i>Saccharomyces cerevisiae</i>	0.1	6

3.4.3 Initial Evaluation of Trial Combinations

After the designated fermentation period for each trial combination, the batter was evaluated for pH, volume rise and bulk density, as described in Sections 3.3.1.3, 3.3.1.4, and 3.3.1.5, respectively. Freshly steamed idlis were prepared from each batch by filling the fermented batter into greased idli moulds and steaming for 10–12 minutes at 100 °C until fully cooked. Sensory evaluation of the steamed idlis was performed using a 9-point hedonic scale by a semi-trained panel for colour, appearance, texture, flavour/aroma, taste, mouthfeel, aftertaste, and overall acceptability. The data obtained from all 17 trials were tabulated, compared, and statistically analysed to identify the most promising combinations for further investigation.

3.4.4 Selection of Best Combinations

From the initial evaluation of 17 experimental trials, five combinations—including the control—were shortlisted for the shelf-life study. Selection was based on a composite assessment of fermentation activity (volume rise, bulk density, pH decline), physico-chemical stability, and sensory acceptability scores.

3.5 SHELF-LIFE STUDIES OF STANDARDIZED IDLI BATTER

Following the selection of the best-performing starter culture combinations from the initial evaluation trials, the standardized idli batter was subjected to a comprehensive shelf-life study to assess the changes in its physico-chemical, microbiological, and sensory quality attributes during refrigerated storage. The shelf-life study is an essential component of food product development, as it establishes the period during which the product retains its safety, stability, and sensory acceptability within specified storage conditions.

Fresh idli batter was prepared for each of the selected treatment combinations as described in Section 3.2. Portions of 100 g of the freshly prepared batter were filled in LDPE pouches and hermetically sealed using a laboratory induction sealer to prevent moisture loss and contamination during storage. All pouches were appropriately labelled with the treatment code, date of preparation, and storage condition. The sealed pouches were stored under refrigerated conditions at 4 ± 1 °C throughout the study period, as refrigeration is the most commonly adopted preservation strategy for extending the shelf life of fermented foods by suppressing the growth of mesophilic spoilage organisms while partially slowing lactic acid bacterial activity.

Quality evaluation was conducted at four designated storage intervals: the 0th day (freshly prepared batter, serving as the baseline), 7th day, 14th day, and 21st day. At each interval, individual pouches were removed from the refrigerator, allowed to equilibrate to room temperature for 30 minutes, and analysed for physico-chemical properties. Fresh idlis were steamed from the stored batter samples and subjected to sensory evaluation. Microbiological quality of the batter was assessed by total plate count at each storage interval to monitor microbial proliferation and ensure compliance with food safety standards.

3.5.1 Quality Evaluation of Stored Samples

3.5.1.1 Physico-Chemical Tests

The physico-chemical quality parameters monitored during the shelf-life study included pH, titratable acidity, colour (L^* , a^* , b^*), bulk density, and volume rise. pH measurements were carried out using the digital pH meter as described in Section 3.3.1.3. The decrease in pH over the storage period reflects the continued production of organic acids by residual lactic acid bacteria present in the refrigerated batter, albeit at a reduced rate compared to fermentation at optimum temperature. Titratable acidity was determined using the titration method described in Section 3.3.1.2, and the progressive increase in TA during storage was used to assess the rate of acid accumulation and the threshold beyond which the batter becomes unacceptably sour.

Colour values (L^* , a^* , b^*) were recorded using the Lovibond colourimeter as described in Section 3.3.1.1. Changes in L^* and b^* values during storage were used to monitor the development of browning or discolouration in the batter, which may occur due to Maillard reactions, oxidation of phenolic compounds, or microbial pigment production. Bulk density and volume rise were measured as described in Sections 3.3.1.4 and 3.3.1.5, respectively, to assess changes in the structural and fermentative properties of the batter during the storage period. A gradual increase in bulk density and decrease in volume rise during storage would indicate loss of fermentation potential and reduction in batter aeration over time.

3.5.1.2 Sensory Evaluation

Sensory evaluation is a scientific discipline that applies principles of experimental design and statistical analysis to the use of human senses of sight, smell, taste, touch, and hearing to measure, analyze, and interpret the characteristics of food products. It constitutes an indispensable component of food quality assessment, as instrumental analyses, while objective and precise,

cannot fully capture the complex, multidimensional sensory experience of the consumer. In the context of fermented foods such as idli, sensory evaluation provides direct and consumer-relevant information on the effect of starter culture type, fermentation conditions, and storage duration on the perceived quality of the product.

Sensory evaluation of freshly steamed idli prepared from stored batter was conducted by a semi-trained panel of eight judges comprising assistant professors of Kelappaji College of Agricultural Engineering and Food Technology (KCAEFT), Tavanur, who were familiar with the sensory characteristics of standard idli. The panelists were briefed on the evaluation procedure and scoring criteria before each session. Fresh idlis were prepared by steaming the stored batter in idli moulds for 10–12 minutes and presented to the panel in clean, labelled containers bearing randomized three-digit codes to prevent identification bias.

The samples were evaluated for eight quality attributes: colour, appearance, texture, flavour/aroma, taste, mouthfeel, aftertaste, and overall acceptability. A 9-point hedonic scale was used for scoring, ranging from 1 (Dislike Extremely) to 9 (Like Extremely), as outlined in Table 3.7. Sensory evaluation sessions were conducted separately for each storage interval—0th day, 7th day, 14th day, and 21st day—to track changes in acceptability over the storage period. Panelists were provided with plain water and unsalted crackers to cleanse their palate between samples. The evaluation was carried out in individual booths under adequate lighting conditions to minimize distractions and inter-panelist interaction.

RESULT AND DISCUSSIONS

CHAPTER IV

RESULT AND DISCUSSION

This chapter presents and critically discusses the findings obtained from the standardization of idli fermentation using selected starter culture combinations. The effects of starter culture composition, inoculum concentration and fermentation time on the physico-chemical, microbiological, sensory and storage characteristics of idli batter were systematically evaluated. The results are interpreted in the context of fermentation mechanisms and supported by relevant scientific literature to identify the optimum process conditions for producing high-quality, standardized idli batter.

4.1. PHYSICO-CHEMICAL PROPERTIES OF FRESH IDLI BATTER

The idli batter was prepared according to the standardized procedure described in Section 3.2. The physico-chemical properties of freshly prepared idli batter were evaluated prior to the incorporation of the selected starter culture. The freshly prepared batter was analysed for selected quality parameters, namely colour, titratable acidity, pH and bulk density. These parameters were assessed to establish the initial physico-chemical characteristics of the batter before subjecting it to controlled fermentation using the selected starter culture. The results obtained for the fresh idli batter are presented in Table 4.1

Table 4.1. Process Parameters of Fresh Idli Batter

Sl. No.	Parameter	Value
1	Colour (L* value)	84.3
2	Titratable acidity	0.42
3	pH	5.8
5	Bulk density	0.92

The fresh batter recorded a colour (L* value) of 84.3, with a titratable acidity of 0.42 and a pH of 5.8, indicating the initial physico-chemical condition of the batter. The batter exhibited a bulk density of 0.92.

Following the assessment of the fresh batter, selected starter culture combinations were inoculated at different concentrations and subjected to different fermentation periods according to the experimental design. Three independent variables were selected for the standardization of idli fermentation: microbial culture, microbial culture concentration, and fermentation period. Microbial culture was considered at three levels, namely LY (*Lactococcus lactis* + *Saccharomyces cerevisiae*), LPF (*Lactococcus lactis* + *Lactobacillus plantarum* + *Lactobacillus fermentum*), and LP (*Lactococcus lactis* + *Lactobacillus plantarum*). The microbial culture concentration was varied at three levels (0.1, 0.2, and 0.3%), while the fermentation period was varied at three levels (2, 4, and 6 h). The inoculated batter was incubated for the predetermined fermentation periods. The experimental design and statistical analysis were carried out using Design-Expert software to evaluate the effects of the independent variables on the selected response variables and to identify the optimum fermentation conditions. Following fermentation, the batter samples were systematically evaluated for their physico-chemical, microbiological and sensory characteristics to assess the influence of the selected starter culture combinations and to identify the optimum process conditions. The findings obtained from these evaluations are presented and discussed in the subsequent sections.

4.2. OPTIMIZATION OF PROCESS PARAMETERS FOR IDLI FERMENTATION

4.2.1 Effect of Process Parameters on Colour Characteristics during fermentation

Colour is one of the most important quality attributes influencing the consumer acceptability of fermented idli batter. The colour of the batter was evaluated using the Tintometer, Lovibond® colour measurement instruments to determine the stability of starter culture formulations in comparison with the control during fermentation. Changes in these colour parameters reflect physico-chemical alterations occurring during fermentation due to continued microbial metabolism and biochemical reactions. The results indicated that the L* values of idli batter gradually decreased with increasing fermentation time and starter culture concentration, suggesting a slight reduction in batter lightness as fermentation progressed. The control sample exhibited the highest L* value of 84.3 after 6 hours of fermentation, indicating the brightest appearance among all treatments. In contrast, samples inoculated with starter cultures showed progressively lower L* values depending on the duration and intensity of fermentation. Treatments fermented for 2 hours exhibited L* values ranging from 80.4 to 84.1, while those fermented for 4

hours ranged between 79.6 and 84.2. The greatest reduction in lightness was observed after 6 hours of fermentation, where the LPF treatment (0.3 ppm) recorded an L* value of 78.4, followed closely by LP treatment (0.2 ppm) with an L* value of 78.7. The LY combination fermented for 6 hours produced the lowest L* value (76.9), indicating comparatively greater darkening during fermentation. Although the LPF 0.3 treatment recorded a slightly higher b* value at the end of fermentation, the difference was not visually perceptible, demonstrating that the optimized starter culture effectively maintained the colour stability of the batter during fermentation.

Table 4.2. Colour parameters of fermented idli batter

SI NO.	COMBINATION	CONCENTRATION	TIME	COLOUR
1	Control			84.3
2	LY	0.1	2	84.1
3	LPF	0.2	2	82.5
4	LP	0.2	2	83.7
5	LY	0.3	2	80.4
6	LP	0.3	4	81.1
7	LPF	0.1	4	84.2
8	LPF	0.3	4	79.6
9	LY	0.2	4	82.2
10	LP	0.1	4	83.1
11	LPF	0.3	6	78.4
12	LY	0.3	6	76.9
13	LP	0.2	6	78.7
14	LY	0.1	6	77.3

The colour characteristics of fermented idli batter are presented in Table 4.1. The CIE colour parameters (L^* , a^* , and b^*) exhibited only minor variations throughout the fermentation period, indicating good colour stability under fermentation conditions.

Among the starter culture combinations evaluated, the LPF consortium demonstrated superior colour stability despite prolonged fermentation. The presence of *Lactococcus lactis*, *Lactobacillus plantarum*, and *Lactobacillus fermentum* appears to have promoted balanced fermentation, preventing excessive biochemical reactions that could otherwise result in undesirable darkening. The LY combinations exhibited relatively lower L^* values at longer fermentation periods, suggesting that yeast-assisted fermentation may have accelerated certain metabolic reactions that slightly increased colour loss. Nevertheless, the observed changes remained within the acceptable range for fermented idli batter. Fermentation time had a greater impact on colour stability than inoculum concentration, with the optimized treatments maintaining acceptable visual quality even after 6 h of fermentation. These findings further support the application of selected starter cultures as an effective strategy for achieving consistent product quality while preserving the desirable visual characteristics of fermented idli batter

The findings of the present study are consistent with previous reports on cereal-based fermented foods. Sridevi *et al.*, (2022) observed that controlled lactic acid bacterial fermentation caused only a slight reduction in batter lightness due to starch hydrolysis and organic acid production while maintaining the characteristic cream-white appearance of fermented batters. Similarly, Roy *et al.*, (2023) reported that minor changes in L^* values during controlled fermentation reflected normal biochemical modifications rather than pigment degradation. Sharma *et al.*, (2023) further demonstrated that defined lactic acid bacterial starter cultures preserved colour more effectively than spontaneous fermentation by minimizing oxidative reactions and reducing pigment degradation during fermentation.

The influence of process parameters on the colour characteristics of fermented idli batter was evaluated using analysis of variance (ANOVA) to determine the statistical significance of inoculum concentration (A), fermentation time (B), and starter culture combination (C) on the colour response. The ANOVA results are presented in Table 4.2. The fitted quadratic model was highly significant ($p < 0.0001$), indicating that the selected model adequately described the relationship between the independent variables and colour values. Furthermore, the non-significant

lack-of-fit ($p = 0.1726$) confirmed the suitability of the model for predicting colour changes within the experimental range.

Table 4.3. Analysis of variance (ANOVA) for Colour analysis

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	143.63	11	13.06	9.45	< 0.0001	significant
A-Concentration	6.6	1	6.6	4.78	0.0377	
B-Time	107.56	1	107.56	77.84	< 0.0001	
C-Organism	0.1605	2	0.0803	0.0581	0.9437	
AB	4.08	1	4.08	2.96	0.0971	
AC	4.95	2	2.48	1.79	0.1858	
BC	2.05	2	1.02	0.741	0.4861	
A ²	5.99	1	5.99	4.33	0.047	
B ²	5.3	1	5.3	3.84	0.0605	
Residual	37.31	27	1.38			
Lack of Fit	25.5	15	1.7	1.73	0.1726	not significant
Pure Error	11.81	12	0.984			
Cor Total	180.94	38				

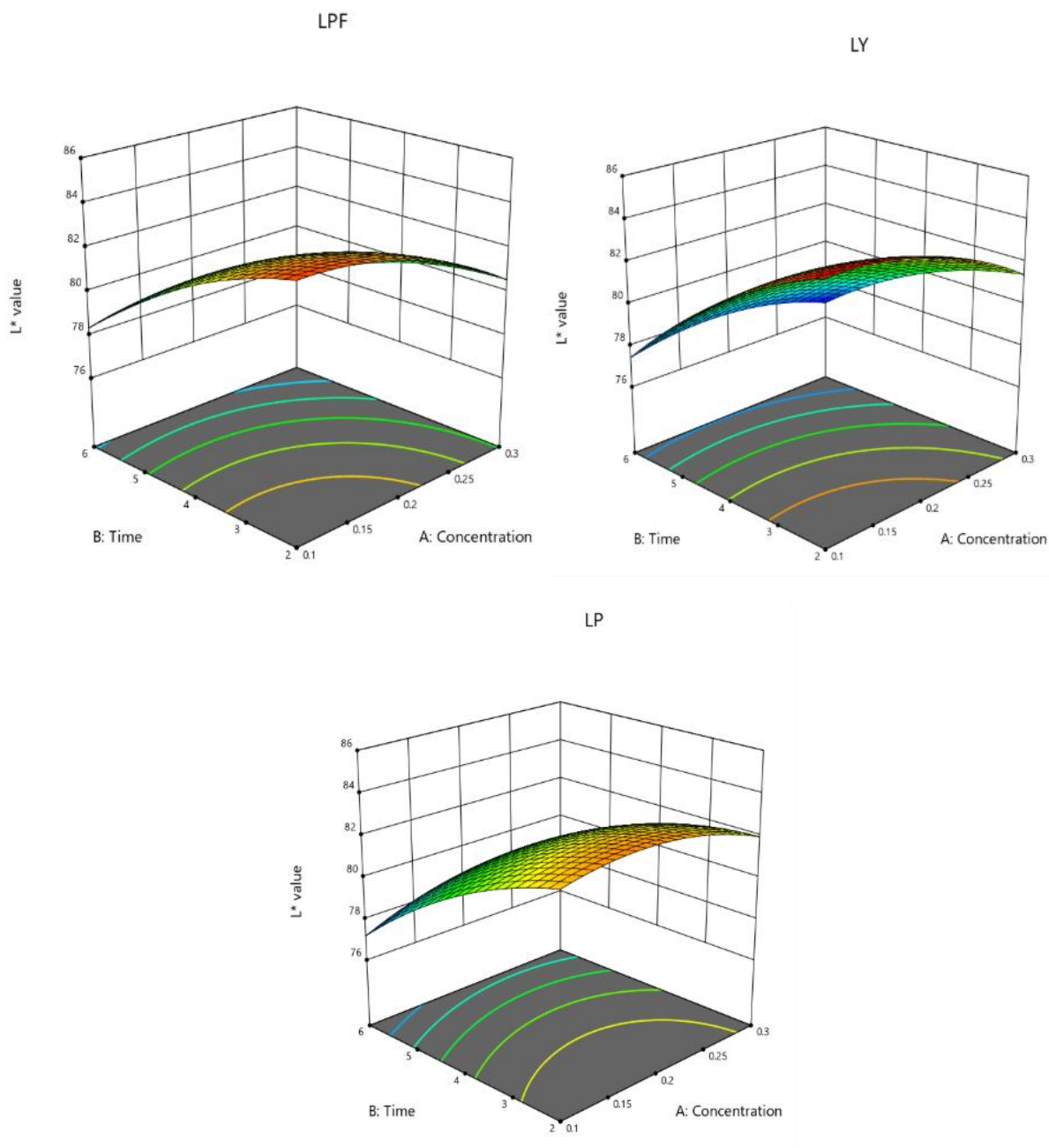


Fig.4.1. Effect of process parameter of colour analysis

The analysis of variance (ANOVA) revealed that the fitted quadratic model for colour analysis was highly significant ($p < 0.0001$), indicating that the selected model adequately described the relationship between the process variables and the colour characteristics of fermented idli batter. The non-significant lack-of-fit ($p = 0.1726$) confirmed that the regression

model was suitable for predicting colour changes in fermented idli batter under the selected experimental conditions.

Among the process variables, fermentation time (Factor B) had the greatest influence on colour, as indicated by the highest F-value (77.84) and a highly significant p-value (<0.0001). Increasing fermentation time caused a slight reduction in lightness (L^*) and minor changes in the a^* and b^* values due to continued microbial metabolism and organic acid production. However, these changes remained within acceptable limits, preserving the characteristic cream-white appearance of the batter. Starter culture concentration (Factor A) also significantly influenced colour ($p = 0.0377$), indicating that increasing inoculum concentration slightly affected colour development during fermentation.

The starter culture combination (Factor C) had no significant effect on colour ($p = 0.9437$). Likewise, the interaction effects (AB, AC and BC) were not significant ($p > 0.05$), indicating that the combined influence of the process variables did not significantly alter the colour characteristics of the batter. Among the quadratic terms, only A^2 showed a significant effect ($p = 0.0470$), whereas B^2 was not significant ($p = 0.0605$), suggesting that colour changes occurred almost linearly over the fermentation period.

Overall, the statistical analysis demonstrated that fermentation time was the most influential factor affecting colour stability, followed by starter culture concentration, while the type of starter culture and interaction effects had no significant influence. These findings indicate that the optimized fermentation conditions effectively maintained the desirable colour of fermented idli batter while ensuring consistent product quality.

4.2.2. Effect of Process Parameters on Titratable Acidity during fermentation

Titrateable acidity is an important indicator of idli batter fermentation, as it reflects the total organic acids produced by lactic acid bacteria. Unlike pH, it measures the overall acid content of the batter, making it a reliable indicator of fermentation efficiency, flavour development, and microbial stability. Therefore, monitoring changes in titrateable acidity is essential for evaluating the efficiency of starter cultures and determining the optimum fermentation conditions.

The results of the present study demonstrated that titrateable acidity increased progressively with increasing fermentation time and inoculum concentration. The control sample exhibited the

lowest titratable acidity of 0.42% lactic acid, indicating comparatively limited acid production during spontaneous fermentation. In contrast, all inoculated treatments showed significantly higher acidity, confirming the enhanced metabolic activity of the selected starter cultures. After 2 hours of fermentation, titratable acidity ranged between 0.46 and 0.54% lactic acid, indicating the initiation of organic acid production. With an increase in fermentation time to 4 hours, the acidity further increased, ranging from 0.54 to 0.61% lactic acid. The highest acidity values were observed after 6 hours of fermentation, where the optimized LPF (0.3 ppm) treatment recorded 0.68% lactic acid, followed closely by the LP (0.2 ppm) treatment with 0.65% lactic acid. The LY (0.3 ppm, 6 h) treatment exhibited the highest acidity (0.72% lactic acid), indicating comparatively greater acid production during fermentation.

Table 4.4. Titratable acidity (% lactic acid) of fermented idli batter

SI NO.	COMBINATION	CONCENTRATION	TIME	TITRATABLE ACIDITY
1	Control			0.42
2	LY	0.1	2	0.48
3	LPF	0.2	2	0.45
4	LP	0.2	2	0.52
5	LY	0.3	2	0.5
6	LP	0.3	4	0.6
7	LPF	0.1	4	0.58
8	LPF	0.3	4	0.59
9	LY	0.2	4	0.56
10	LP	0.1	4	0.51
11	LPF	0.3	6	0.68
12	LY	0.3	6	0.7
13	LP	0.2	6	0.65
14	LY	0.1	6	0.62

The progressive increase in titratable acidity reflects the active metabolism of the inoculated lactic acid bacteria, which efficiently utilized fermentable carbohydrates for organic acid production. The increase in acidity was accompanied by a corresponding decrease in pH, confirming successful lactic acid fermentation. Among the starter culture combinations evaluated, the LPF treatment produced the most desirable acidification pattern. The synergistic activity of *Lactococcus lactis*, *Lactobacillus plantarum*, and *Lactobacillus fermentum* resulted in balanced acid production, creating favourable conditions for flavour development, microbial safety, and overall product quality. Although the LY treatment produced the highest acidity (0.72% lactic acid), it received a comparatively lower sensory score (6.0), suggesting that excessive acid production adversely affected flavour through increased sourness. In contrast, the LPF treatment, with 0.68% lactic acid, achieved the highest sensory score (8.8), indicating that moderate acidification is more desirable than maximum acid production.

The present findings agree with recent studies (2021–2026) on starter culture-assisted fermentation of cereal–legume products, which have reported significantly higher titratable acidity in inoculated samples than in spontaneously fermented batters due to enhanced carbohydrate metabolism and lactic acid production. Similar observations were reported by Rani et al. (2023), who found that mixed lactic acid bacterial cultures promoted controlled acidification and improved flavour development. Likewise, Chen *et al.* (2024) emphasized that moderate titratable acidity is essential for maintaining an appropriate balance between microbial safety and sensory quality in fermented cereal products. The acidity values obtained in the present study were comparable with those reported by Roy et al. (2023) (0.57–0.85% lactic acid) for fermented idli batter, while Wijesinghe et al. (2021) observed titratable acidity increasing from 0.24% to 0.92% during fermentation. The moderate acidity achieved in the optimized LPF treatment therefore indicates effective fermentation that enhanced flavour without causing excessive sourness.

The effect of process parameters on the titratable acidity of fermented idli batter was evaluated using analysis of variance (ANOVA), and the results are presented in Table 4.4. The quadratic regression model was found to be highly significant ($p < 0.0001$), indicating that the developed model adequately explained the variation in titratable acidity under the selected

experimental conditions. Furthermore, the lack-of-fit was non-significant ($p = 0.2349$), confirming the adequacy of the model for predicting titratable acidity within the experiment.

Table 4.5. Analysis of Variance (ANOVA) for Titratable Acidity

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.1655	11	0.015	9.9	< 0.0001	significant
A-Concentration	0.02	1	0.02	13.16	0.0012	
B-Time	0.1089	1	0.1089	71.63	< 0.0001	
C-Organism	0.0102	2	0.0051	3.34	0.0505	
AB	0.0003	1	0.0003	0.1973	0.6604	
AC	0.0005	2	0.0003	0.1754	0.8401	
BC	0.0025	2	0.0012	0.8149	0.4533	
A ²	0.0177	1	0.0177	11.64	0.0021	
B ²	0.0003	1	0.0003	0.21	0.6505	
Residual	0.041	27	0.0015			
Lack of Fit	0.0259	15	0.0017	1.37	0.2966	not significant
Pure Error	0.0152	12	0.0013			
Cor Total	0.2066	38				

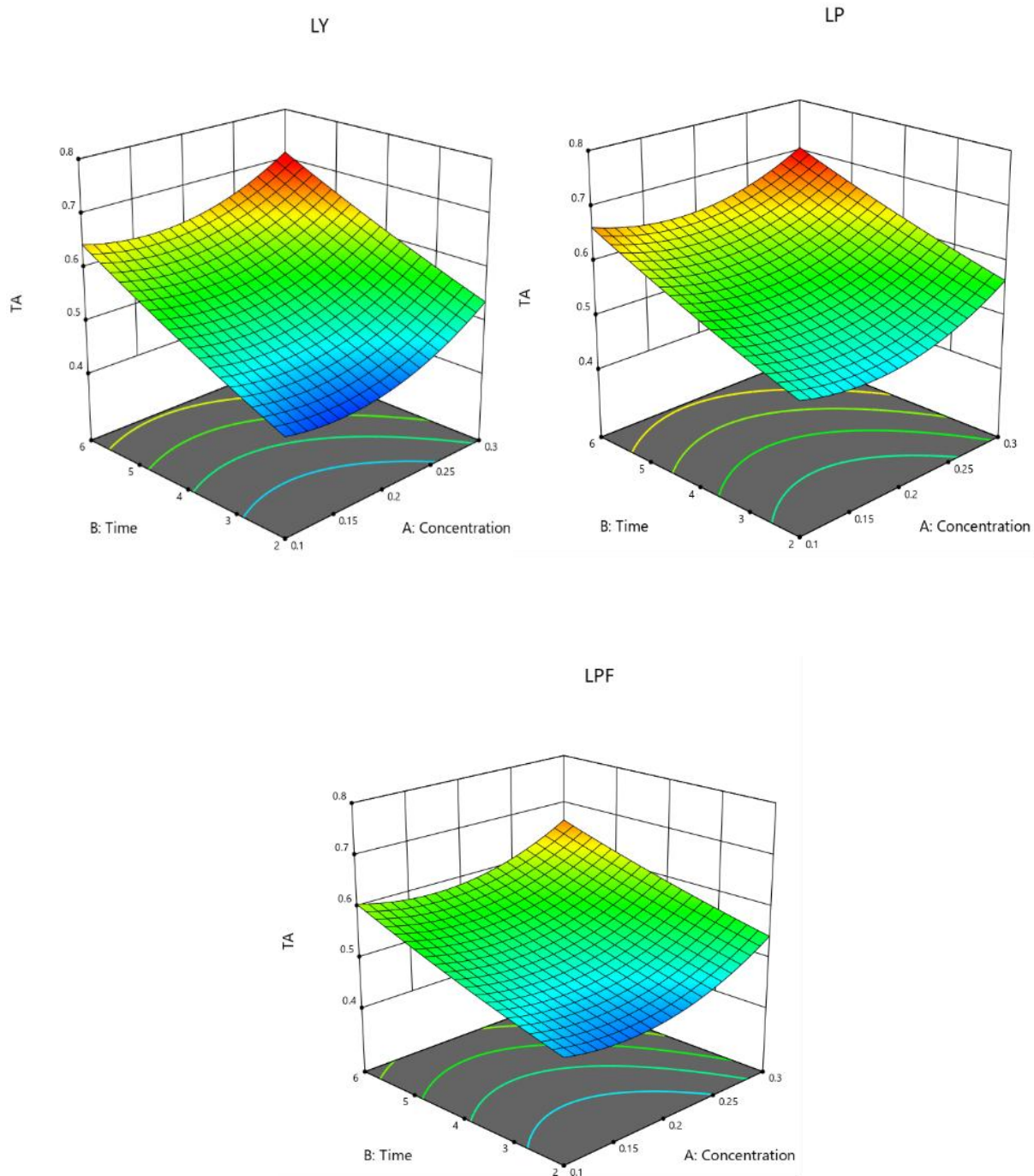


Fig 4.2 Effect of Process Parameters on Titratable Acidity

The ANOVA results revealed that fermentation time (Factor B) had the greatest influence on titratable acidity, as indicated by the highest F-value (143.16) and a highly significant p-value (<0.0001). This demonstrates that fermentation time was the primary factor governing acid production in the idli batter. As fermentation progressed, the metabolic activity of lactic acid

bacteria increased, resulting in greater conversion of fermentable carbohydrates into lactic acid and other organic acids. Consequently, titratable acidity increased significantly with increasing fermentation time.

Starter culture concentration (Factor A) also had a significant effect on titratable acidity ($p = 0.0024$). Increasing the inoculum concentration enhanced microbial growth and fermentation efficiency, thereby promoting greater acid production. The higher acidity observed at increased inoculum levels indicates that starter culture concentration plays an important role in accelerating the fermentation process and improving acidification.

In contrast, the type of starter culture (Factor C) did not significantly influence titratable acidity ($p = 0.2474$). Similarly, the interaction effects between concentration and time (AB), concentration and organism (AC), and time and organism (BC) were statistically non-significant ($p > 0.05$), indicating that the combined effects of these process variables did not significantly alter acid production. These findings suggest that fermentation time and inoculum concentration independently exerted a greater influence on titratable acidity than the interaction among the variables.

Among the quadratic terms, A^2 showed a significant effect ($p = 0.0313$), indicating a slight non-linear relationship between starter culture concentration and titratable acidity. However, the quadratic effect of fermentation time (B^2) was not significant ($p = 0.1954$), suggesting that acid production increased in an approximately linear manner over the fermentation period investigated.

Overall, the statistical analysis demonstrated that fermentation time was the most influential factor affecting titratable acidity, followed by starter culture concentration, whereas the starter culture type and interaction effects were not statistically significant. The highly significant regression model together with the non-significant lack-of-fit confirmed the reliability of the developed model for predicting titratable acidity under the fermentation conditions. These results indicate that controlled fermentation time and inoculum concentration are critical for achieving optimum acidification while maintaining the quality and stability of fermented idli batter.

4.2.3 Effect of Process Parameters on pH during fermentation

The pH of fermented idli batter is an important physico-chemical parameter that reflects the progress of fermentation and the extent of organic acid production by lactic acid bacteria. An

optimal pH enhances the characteristic sour flavour, improves batter stability, and inhibits undesirable microorganisms, whereas excessive acidification can lead to over-fermentation and reduce the sensory quality of the final product.

The results demonstrated that the incorporation of defined starter cultures significantly enhanced acidification compared with spontaneous fermentation. The control sample recorded the highest pH (5.8), indicating comparatively lower acid production during natural fermentation. In contrast, all starter culture-treated samples exhibited lower pH values, confirming enhanced metabolic activity of the inoculated LAB. During 2 h of fermentation, pH values ranged from 5.4 to 5.7, indicating the initial stage of acid production. Extending the fermentation period to 4 h further reduced the pH to 5.1–5.4, while 6 h fermentation resulted in the greatest acidification. Among the optimized treatments, LPF (0.3 ppm) and LP (0.2 ppm) recorded pH values of 4.8 and 4.9, respectively, whereas LY (0.3 ppm) exhibited the lowest pH (4.7), indicating the highest acid production. The gradual decline in pH with increasing fermentation time confirms active growth and carbohydrate metabolism by LAB, resulting in continuous lactic acid production and improved fermentation efficiency. The effect of starter cultures, inoculum concentration, and fermentation time on the pH of idli batter is presented in Table 4.6

Table 4.6. pH of Fermented Idli Batter

SI NO.	COMBINATION	CONCENTRATION	TIME	pH
1	Control			5.8
2	LY	0.1	2	5.6
3	LPF	0.2	2	5.7
4	LP	0.2	2	5.4
5	LY	0.3	2	5.5
6	LP	0.3	4	5.1
7	LPF	0.1	4	5.2

8	LPF	0.3	4	5.2
9	LY	0.2	4	5.3
10	LP	0.1	4	5.4
11	LPF	0.3	6	4.8
12	LY	0.3	6	4.7
13	LP	0.2	6	4.9
14	LY	0.1	6	5

Although the LY treatment produced the lowest pH, it did not achieve the highest sensory acceptability, receiving a comparatively lower sensory score (6.0). Excessive acidification is generally associated with increased sourness, which can negatively influence consumer preference. The selected combination of *Lactococcus lactis*, *Lactobacillus plantarum*, and *Lactobacillus fermentum* in the LPF treatment provided balanced acid production, resulting in improved fermentation efficiency, desirable flavour, and overall product quality.

The reduction in pH during both fermentation and storage closely corresponded with the increase in titratable acidity, confirming the inverse relationship between these parameters. Similarly, lower pH values were associated with increased microbial counts and greater batter volume rise, indicating that acid production occurred simultaneously with LAB proliferation and carbon dioxide generation. This controlled acidification not only enhanced fermentation efficiency but also improved microbial safety by suppressing undesirable microorganisms while promoting the dominance of beneficial LAB.

The findings of the present investigation are consistent with previous reports on controlled fermentation of cereal-legume batters. Patel et al. (2022) reported that defined LAB starter cultures accelerated acid production and produced a more rapid decline in pH than spontaneous fermentation. Similarly, Zhang et al. (2023) suggested that maintaining the pH between 4.7 and 5.0 provides an optimum balance between microbial safety, flavour development, and product quality in fermented cereal-based foods. The optimized LPF treatment achieved a pH of 4.8, which

falls within this desirable range while maintaining excellent sensory quality. Comparable pH values have also been reported by Roy *et al.*, (2023) (4.93–5.18) and Wijesinghe *et al.*, (2021), who observed a decline in pH from 6.28 to 3.72 during extended fermentation. In comparison, the present study achieved the desirable pH range within only 6 h of fermentation, demonstrating the efficiency of the selected starter culture combination.

The effect of process parameters on the pH of fermented idli batter was evaluated using analysis of variance (ANOVA), and the results are presented in Table 4.6. The quadratic regression model was found to be highly significant ($p < 0.0001$), indicating that the selected model adequately explained the variation in pH under the experimental conditions. Furthermore, the non-significant lack-of-fit confirmed the adequacy of the model for predicting pH changes within the experimental range.

Table 4.7. Analysis of Variance (ANOVA) for pH

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.63	11	0.1479	10.57	< 0.0001	significant
A-Concentration	0.0022	1	0.0022	0.1587	0.6935	
B-Time	0.98	1	0.98	69.98	< 0.0001	
C-Organism	0.022	2	0.011	0.7851	0.4662	
AB	0.12	1	0.12	8.57	0.0069	
AC	0.0344	2	0.0172	1.23	0.3082	
BC	0.0533	2	0.0267	1.9	0.1684	
A ²	0.0002	1	0.0002	0.0151	0.903	
B ²	0.3484	1	0.3484	24.88	< 0.0001	
Residual	0.3781	27	0.014			
Lack of Fit	0.2895	15	0.0193	2.61	0.0502	not significant

Pure Error	0.0886	12	0.0074			
Cor Total	2.01	38				

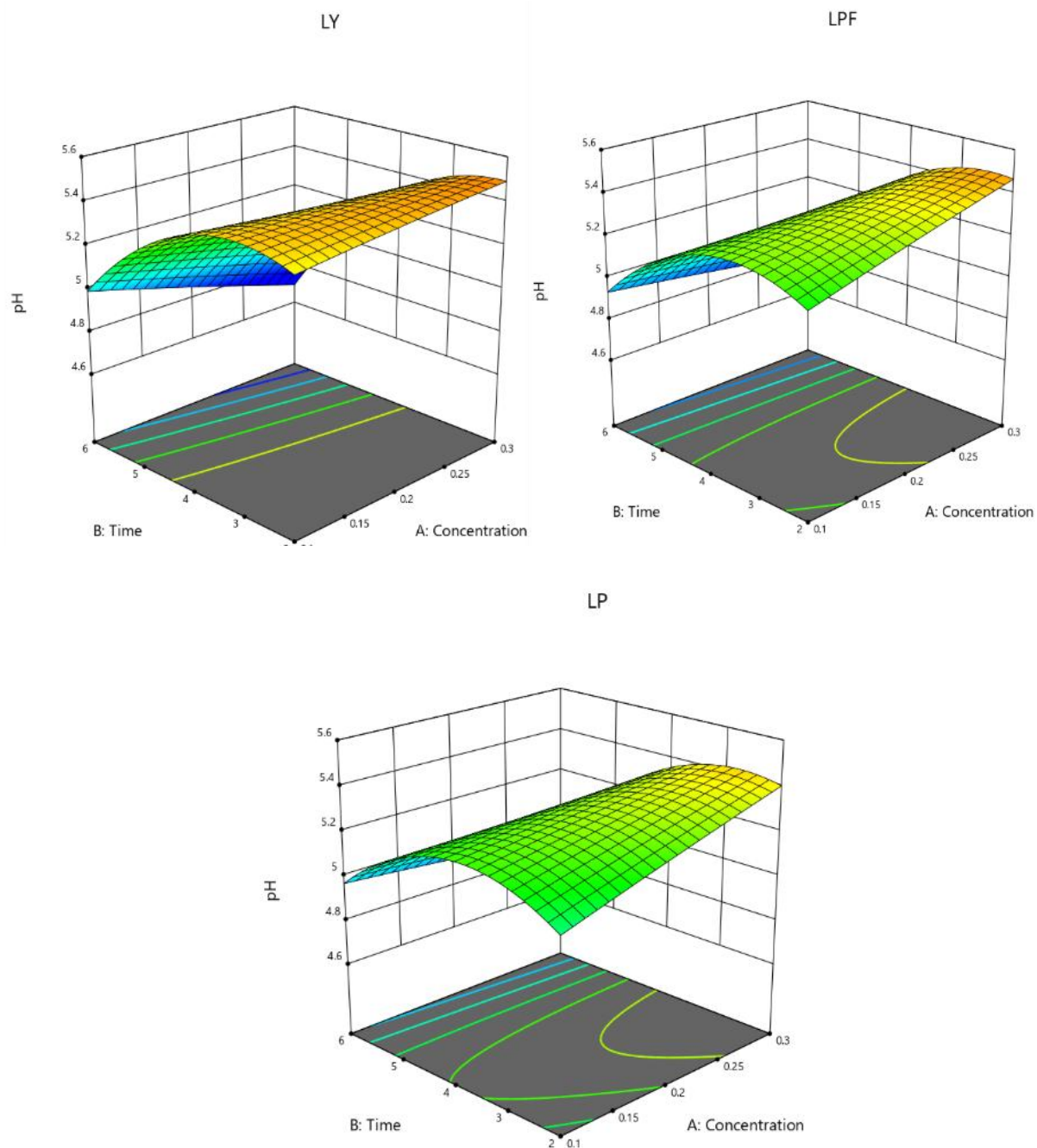


Fig 4.3 Effect of Process Parameters on pH Analysis

4.2.4 Effect of Process Parameters on Volume Rise during fermentation

Volume rise is an important quality indicator of fermented idli batter, reflecting the efficiency of fermentation, carbon dioxide production, and the gas-retention capacity of the batter. Increased volume rise is generally associated with improved aeration, softer texture, greater porosity and enhanced consumer acceptability of the final product.

The results demonstrated that starter culture combinations, inoculum concentration and fermentation time significantly influenced batter expansion. The control sample, which underwent spontaneous fermentation, recorded the lowest volume rise (18 mL), indicating comparatively poor fermentation efficiency. In contrast, inoculation with selected LAB starter cultures markedly enhanced batter expansion.

Table 4.8. Volume Rise (ML) of Fermented Idli Batter

SI NO.	COMBINATION	CONCENTRATION	TIME	VOLUME RISE
1	Control			18
2	LY	0.1	2	22
3	LPF	0.2	2	20
4	LP	0.2	2	26
5	LY	0.3	2	24
6	LP	0.3	4	32
7	LPF	0.1	4	28
8	LPF	0.3	4	30
9	LY	0.2	4	29
10	LP	0.1	4	25
11	LPF	0.3	6	38
12	LY	0.3	6	40
13	LP	0.2	6	34
14	LY	0.1	6	31

During 2 h of fermentation, volume rise ranged from 20 to 26 mL, with LP (0.2 ppm) producing the highest value (26 mL). Increasing the fermentation period to 4 h further improved batter expansion (25–32 mL), while the maximum volume rise was observed after 6 h of fermentation. The eLPF (0.3 ppm) treatment achieved a volume rise of 38 mL, followed by LP (0.2 ppm) (34 mL), whereas the LY (0.3 ppm) treatment recorded the highest numerical value (40 mL).

Although the LY treatment exhibited the greatest batter expansion, it did not produce the highest sensory acceptability, recording a comparatively lower sensory score (6.0). Excessive gas production may weaken the batter structure, resulting in unstable gas retention, over-expansion, and undesirable flavour and texture. In contrast, the LPF (0.3 ppm) treatment achieved the highest sensory score (8.8) by maintaining an optimum balance between carbon dioxide production, batter stability, and controlled acidification. These findings demonstrate that optimum rather than maximum batter expansion is essential for producing high-quality idli.

A close relationship was observed between volume rise and other quality attributes of the batter. Treatments with higher volume rise generally exhibited lower bulk density, indicating greater incorporation and retention of gas within the batter matrix. Similarly, increased batter expansion corresponded with improved sensory characteristics, particularly softness, sponginess, and overall texture.

The present findings agree with previous studies on controlled fermentation of cereal-legume batters. Prabhasankar *et al.*, (2022) reported that mixed LAB starter cultures significantly improved batter expansion through enhanced carbon dioxide production, resulting in softer and more porous fermented products. Similarly, Kumar *et al.*, (2024) demonstrated that heterofermentative LAB contributed substantially to batter leavening by continuous carbon dioxide production, thereby improving the porosity and sponginess of cereal-based fermented foods. Although the LY formulation produced the highest numerical volume rise, its comparatively lower sensory score supports the observations of Singh *et al.*, (2023), who concluded that optimum rather than excessive gas production is necessary to achieve superior sensory quality. The present study further demonstrated that the LPF (0.3 ppm, 6 h) treatment achieved 38 mL batter expansion within only 6 h, whereas previous studies by Roy *et al.*, (2023) and Rajalakshmi *et al.*, (2023) reported higher volume rises only after considerably longer fermentation periods. This highlights

the efficiency of the selected starter culture combination in achieving rapid and controlled fermentation. The effect of process parameters on the volume rise of fermented idli batter was evaluated using analysis of variance (ANOVA), and the results are presented in Table 4.9.

Table 4.9. Analysis of Variance (ANOVA) for Volume Rise

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F-VALUE	P-VALUE	
Model	636	11	57.82	15.26	< 0.0001	significant
A- Concentration	42.32	1	42.32	11.17	0.0024	
B-Time	542.3	1	542.3	143.16	< 0.0001	
C-Organism	11.15	2	5.57	1.47	0.2474	
AB	1.14	1	1.14	0.3012	0.5877	
AC	5.32	2	2.66	0.7026	0.5041	
BC	13.28	2	6.64	1.75	0.1924	
A ²	19.54	1	19.54	5.16	0.0313	
B ²	6.68	1	6.68	1.76	0.1954	
Residual	102.28	27	3.79			
Lack of Fit	67.03	15	4.47	1.52	0.2349	not significant
Pure Error	35.25	12	2.94			
Cor Total	738.28	38				

The regression model was highly significant ($p < 0.0001$), indicating that the selected process variables adequately explained the variation in volume rise during fermentation.

Furthermore, the non-significant lack-of-fit ($p = 0.2349$) confirmed that the developed model was suitable for predicting the response within the experimental range.

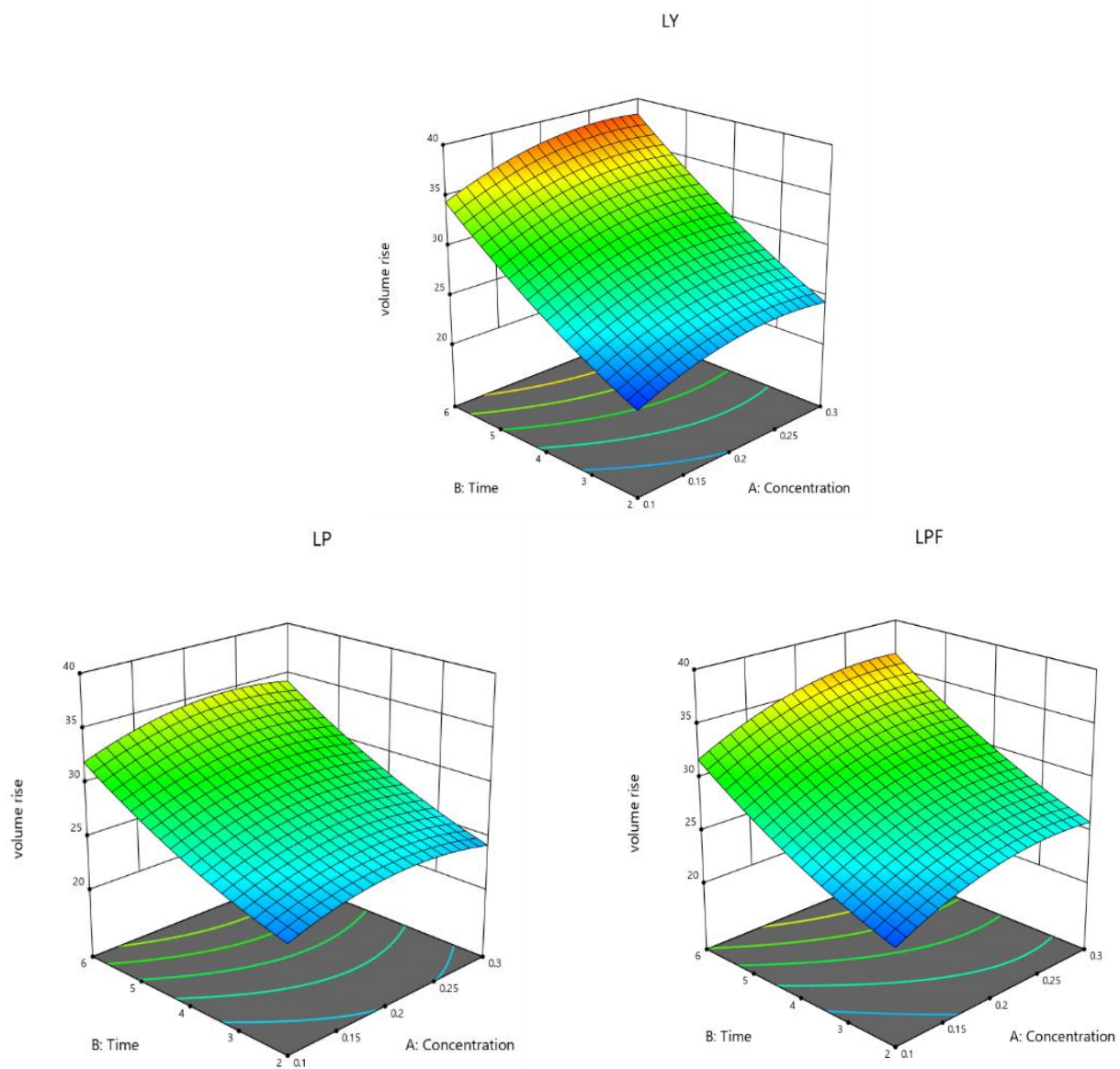


Fig 4.4 Effect of process parameters on volume rise

4.2.5 Effect of Process Parameters on Bulk Density during fermentation

Bulk density indicates the level of aeration in fermented idli batter. During fermentation, carbon dioxide becomes trapped in the batter, increasing its volume and reducing its density. A

lower bulk density indicates greater incorporation and retention of carbon dioxide within the batter matrix, resulting in a lighter, softer, and more porous idli.

The results demonstrated that starter culture combinations, inoculum concentration, and fermentation time significantly influenced the bulk density of idli batter. The control sample exhibited the highest bulk density (0.92 g/mL), indicating limited batter expansion during spontaneous fermentation. In contrast, starter culture-treated samples showed a progressive reduction in bulk density with increasing fermentation time, reflecting enhanced microbial activity, carbon dioxide production, and batter aeration. During 2 h of fermentation, bulk density ranged from 0.88 to 0.84 g/mL. Extending the fermentation period to 4 h further reduced the values to 0.82–0.78 g/mL, while the lowest bulk densities were observed after 6 h of fermentation. Among the treatments, LPF (0.3 ppm) recorded the lowest bulk density (0.72 g/mL), followed by LP (0.2 ppm) (0.75 g/mL), whereas the LY (0.3 ppm) treatment exhibited the lowest numerical value (0.70 g/mL).

The reduction in bulk density with fermentation time reflects the continued production and retention of carbon dioxide within the batter matrix, resulting in increased batter expansion. Although the LY treatment exhibited the lowest numerical bulk density, it recorded a comparatively lower sensory score (6.0), indicating that excessive gas production may weaken the batter structure, reduce gas cell stability, and adversely affect the texture of the steamed idli. In contrast, the LPF (0.3 ppm) treatment achieved a slightly higher bulk density (0.72 g/mL) while recording the highest sensory score (8.8), demonstrating that controlled aeration and stable gas retention are more desirable than excessive expansion. Bulk density exhibited a clear inverse relationship with volume rise. Treatments that showed greater batter expansion consistently recorded lower bulk density, confirming improved incorporation and retention of carbon dioxide within the batter matrix. Likewise, lower bulk density was associated with improved sensory characteristics, particularly softness, porosity, and sponginess of the steamed idli. The LPF and LP formulations consistently combined lower bulk density, higher volume rise, and superior sensory acceptability, demonstrating that efficient gas production and stable batter structure are essential for producing high-quality fermented products.

Table 4.10. Bulk Density (G/ML) of Fermented Idli Batter

SI NO.	COMBINATION	CONCENTRATION	TIME	BULK DENSITY
1	Control			0.92
2	LY	0.1	2	0.88
3	LPF	0.2	2	0.9
4	LP	0.2	2	0.84
5	LY	0.3	2	0.86
6	LP	0.3	4	0.78
7	LPF	0.1	4	0.8
8	LPF	0.3	4	0.79
9	LY	0.2	4	0.81
10	LP	0.1	4	0.85
11	LPF	0.3	6	0.72
12	LY	0.3	6	0.7
13	LP	0.2	6	0.75
14	LY	0.1	6	0.77

The present findings are consistent with previous studies on controlled fermentation of cereal-legume batters. Nagaraju *et al.*, (2022) reported that mixed lactic acid bacterial starter cultures enhanced carbon dioxide retention, reduced batter density, and improved the softness and porosity of steamed fermented products. Similarly, Lee *et al.*, (2024) demonstrated that controlled fermentation reduced batter density while maintaining structural stability. Comparable observations were also reported by Roy *et al.*, (2023), who found that greater air entrapment during fermentation resulted in lower bulk density due to enhanced carbon dioxide production. In the present study, the LPF (0.3 ppm) treatment achieved a bulk density of 0.72 g/mL, representing approximately a 22% reduction compared with the control (0.92 g/mL), thereby confirming the effectiveness of the selected starter culture in producing a well-aerated batter suitable for soft and spongy idli.

Overall, the results demonstrate that fermentation time was the primary factor influencing bulk density, while the LPF (0.3 ppm, 6 h) treatment provided the most desirable balance between batter aeration, structural stability, and sensory quality. The incorporation of selected lactic acid bacterial starter cultures therefore represents an effective strategy for producing standardized idli batter with improved physical characteristics, enhanced fermentation efficiency, and superior consumer acceptability.

The effect of process parameters on the bulk density of fermented idli batter was evaluated using analysis of variance (ANOVA), and the results are presented in Table 4.10. The ANOVA results revealed that the regression model was highly significant ($F = 10.27$; $p < 0.0001$), indicating that the selected model adequately explained the variation in bulk density under the experimental conditions. Furthermore, the non-significant lack-of-fit ($p = 0.1245$) confirmed that the developed regression model was appropriate for predicting bulk density within the investigated experimental range.

Table 4.11. Analysis of variance (ANOVA) for bulk density

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0659	11	0.006	10.27	< 0.0001	significant
A-Concentration	0.0001	1	0.0001	0.0857	0.772	
B-Time	0.045	1	0.045	77.13	< 0.0001	
C-Organism	0.0038	2	0.0019	3.28	0.0532	
AB	0.007	1	0.007	12.01	0.0018	
AC	0.002	2	0.001	1.74	0.1942	
BC	0.0019	2	0.0009	1.63	0.2149	
A ²	0.0006	1	0.0006	1.09	0.3052	
B ²	0.0035	1	0.0035	5.93	0.0218	

Residual	0.0158	27	0.0006			
Lack of Fit	0.0112	15	0.0007	1.95	0.1245	not significant
Pure Error	0.0046	12	0.0004			
Cor Total	0.0817	38				

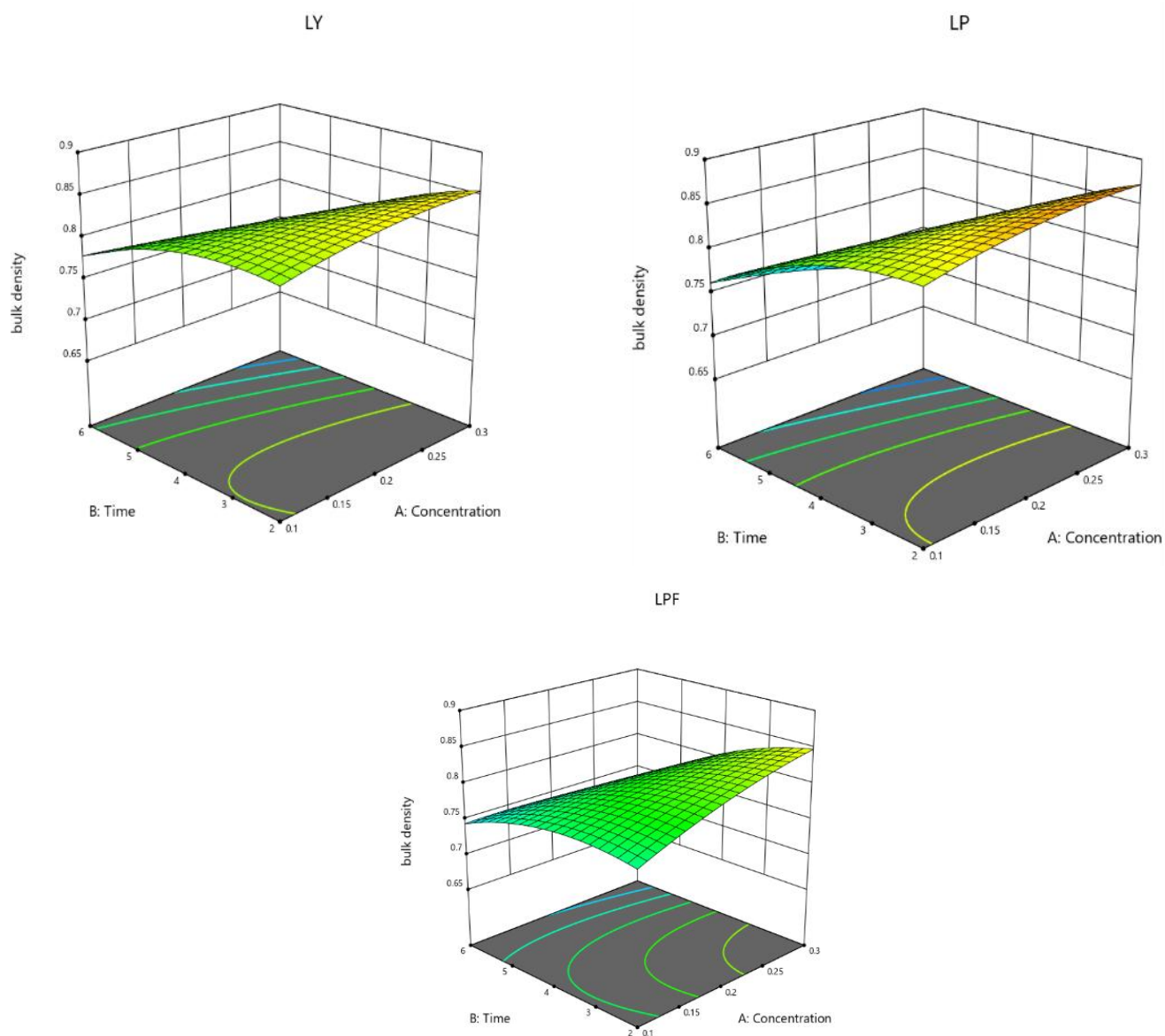


Fig 4.5 Effect of process parameters on bulk density

The ANOVA results indicated that fermentation time (Factor B) had the greatest influence on the bulk density of fermented idli batter, as evidenced by the highest F-value (77.13) and a highly significant p-value (<0.0001). This finding demonstrates that fermentation duration played a dominant role in determining batter density. As fermentation progressed, increased microbial activity resulted in greater production of carbon dioxide, which became entrapped within the batter matrix, thereby increasing batter aeration and reducing bulk density. The reduction in bulk density reflects improved gas retention and contributes to the production of soft and spongy idli.

In contrast, starter culture concentration (Factor A) did not significantly influence bulk density ($p = 0.7720$), indicating that variations in inoculum concentration within the selected range had little effect on the density of the fermented batter. Similarly, the type of starter culture (Factor C) was also not statistically significant ($p = 0.0532$), suggesting that the different microbial combinations produced comparable bulk density values under the experimental conditions.

Among the interaction terms, only the interaction between starter culture concentration and fermentation time (AB) showed a significant effect ($p = 0.0018$), indicating that the influence of fermentation time on bulk density depended on the level of inoculum concentration. However, the interaction effects between concentration and organism (AC) and time and organism (BC) were not statistically significant ($p > 0.05$), demonstrating that these combinations did not produce additional changes in bulk density beyond their individual effects.

Regarding the quadratic terms, B^2 exhibited a significant effect ($p = 0.0218$), indicating a slight non-linear relationship between fermentation time and bulk density. However, the quadratic effect of starter culture concentration (A^2) was not significant ($p = 0.3052$), suggesting that the effect of inoculum concentration on bulk density remained approximately linear within the investigated range.

The non-significant lack-of-fit ($p = 0.1245$) confirmed that the regression model adequately represented the experimental data and could reliably predict bulk density under the selected fermentation conditions. Overall, the statistical analysis demonstrated that fermentation time was the most influential factor affecting bulk density, while starter culture concentration alone and starter culture type had no significant effect. The significant AB interaction further indicates that the combined effect of fermentation time and inoculum concentration plays an important role in determining batter density. These findings suggest that optimizing fermentation time together with

appropriate starter culture concentration is essential for producing fermented idli batter with lower bulk density, improved aeration, and superior textural quality.

4.3 MICROBIOLOGICAL ANALYSIS OF FERMENTED IDLI BATTER

4.3.1 Effect of Process Parameters on Total Plate Count (TPC) during fermentation

Microbiological quality is an important indicator of the safety and quality of fermented idli batter. During fermentation, the growth of beneficial lactic acid bacteria produces organic acids, flavour compounds, and carbon dioxide, which contribute to batter quality. Total Plate Count (TPC) is commonly used to measure the viable microbial population and assess fermentation efficiency. A controlled increase in TPC indicates successful fermentation and improved product quality. Therefore, monitoring the microbial population is essential for evaluating both fermentation efficiency and product quality.

The results of the present study revealed that the total plate count increased progressively with the use of starter cultures and longer fermentation periods. The control sample, which underwent spontaneous fermentation without inoculated starter cultures, recorded the lowest microbial count of 7.2 log CFU/g, indicating comparatively slower microbial proliferation. In contrast, all inoculated treatments exhibited significantly higher microbial populations, confirming the effectiveness of the selected starter cultures in establishing rapid fermentation. After 2 hours of fermentation, the microbial count ranged from 7.4 to 7.9 log CFU/g, reflecting the initial growth phase of the inoculated microorganisms. With increasing fermentation time to 4 hours, the microbial population further increased to 7.9–8.3 log CFU/g, demonstrating active microbial multiplication and metabolic activity. The highest microbial counts were observed after 6 hours of fermentation, where the LPF (0.3 ppm) treatment recorded 8.7 log CFU/g, followed closely by the LP (0.2 ppm) treatment with 8.5 log CFU/g. The LY (0.3 ppm, 6 h) treatment also showed a relatively high microbial count of 8.6 log CFU/g, indicating vigorous microbial growth during fermentation.

The LPF treatment demonstrated the highest desirable microbial growth, indicating the successful establishment of a balanced microbial ecosystem. The synergistic interaction among *Lactococcus lactis*, *Lactobacillus plantarum*, and *Lactobacillus fermentum* contributed significantly to this response. *Lactococcus lactis* rapidly initiated fermentation by producing lactic

acid, creating favourable conditions for the growth of *Lactobacillus plantarum* and *Lactobacillus fermentum*. Together, these cultures established a stable and dominant beneficial microbial population, supporting efficient fermentation and flavour development. The combined action of these microorganisms resulted in a stable and dominant beneficial microbial population throughout the fermentation period.

Table 4.12. Total Plate Count (TPC) Of Fermented Idli Batter

SI NO.	COMBINATION	CONCENTRATION	TIME	TPC
1	Control			7.2
2	LY	0.1	2	7.5
3	LPF	0.2	2	7.4
4	LP	0.2	2	7.6
5	LY	0.3	2	7.5
6	LP	0.3	4	8.1
7	LPF	0.1	4	8
8	LPF	0.3	4	8.2
9	LY	0.2	4	8.1
10	LP	0.1	4	7.9
11	LPF	0.3	6	8.7
12	LY	0.3	6	8.9
13	LP	0.2	6	8.5
14	LY	0.1	6	8.6

The microbiological findings of the present investigation are consistent with recent studies on starter culture-assisted fermentation of cereal-legume batters. Recent research has demonstrated that inoculation with selected lactic acid bacterial cultures significantly accelerates microbial proliferation compared with spontaneous fermentation, leading to faster acidification, improved

fermentation efficiency, and enhanced product consistency. The increase in total plate count observed during fermentation reflects the successful growth of beneficial lactic acid bacteria rather than microbial contamination. The LPF formulation exhibited the highest microbial count, indicating efficient establishment of the starter culture consortium. Similar observations were reported by Ghosh *et al.* (2022), who demonstrated that defined starter cultures rapidly dominate the microbial ecosystem and suppress undesirable microorganisms through organic acid and bacteriocin production. Fernandes *et al.*, (2024) also reported that mixed lactic acid bacterial cultures improve microbial stability and fermentation consistency in cereal-based foods. The microbial population increased from 7.2 log CFU/g in the control to 8.7 log CFU/g in the LPF treatment, indicating successful proliferation of beneficial lactic acid bacteria during fermentation. A similar increase in microbial population was reported by Wijesinghe *et al.*, (2021), who observed LAB counts increasing to 9.88 log CFU/g after 12 hours of fermentation before declining during prolonged fermentation because of excessive acid accumulation. The slightly lower microbial count observed in the present study is expected because fermentation was terminated after 6 hours, whereas the previous study evaluated longer fermentation periods. The results therefore demonstrate efficient microbial growth while preventing over-fermentation. The present findings therefore confirm that higher populations of beneficial microorganisms contribute positively to fermentation efficiency, product safety, and shelf-life stability. These observations closely support the results obtained in the present study.

Overall, the results clearly demonstrate that starter culture combinations significantly enhanced the microbial population of idli batter during fermentation. The increase in total plate count reflected the successful growth of beneficial lactic acid bacteria and was closely associated with improvements in acidification, batter expansion, and sensory quality. Among all treatments, the LPF combination at 0.3 ppm fermented for 6 hours exhibited the most desirable microbial profile, recording a total plate count of 8.7 log CFU/g. These findings confirm that controlled inoculation with selected lactic acid bacterial starter cultures promotes efficient fermentation, enhances microbiological stability, and contributes to the production of standardized idli batter with superior quality and improved commercial potential.

The effect of process parameters on the total plate count (TPC) of fermented idli batter was evaluated using analysis of variance (ANOVA), and the results are presented in Table 4.13. The

ANOVA results revealed that the regression model was highly significant ($F = 10.05$; $p < 0.0001$), indicating that the selected model adequately explained the variation in total plate count during fermentation. Furthermore, the non-significant lack-of-fit ($p = 0.0930$) confirmed that the regression model provided an adequate fit to the experimental data and could reliably predict the microbial response within the selected experimental range.

Table 4.13. Analysis of variance (ANOVA) for total plate count

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9.02	11	0.8196	10.05	< 0.0001	significant
A-Concentration	0.6806	1	0.6806	8.34	0.0075	
B-Time	4.23	1	4.23	51.91	< 0.0001	
C-Organism	2.17	2	1.08	13.29	< 0.0001	
AB	0.0833	1	0.0833	1.02	0.3211	
AC	0.4578	2	0.2289	2.81	0.0781	
BC	0.5791	2	0.2896	3.55	0.0428	
A ²	0.1672	1	0.1672	2.05	0.1637	
B ²	0.3439	1	0.3439	4.22	0.0498	
Residual	2.2	27	0.0816			
Lack of Fit	1.61	15	0.1071	2.16	0.093	not significant
Pure Error	0.596	12	0.0497			
Cor Total	11.22	38				

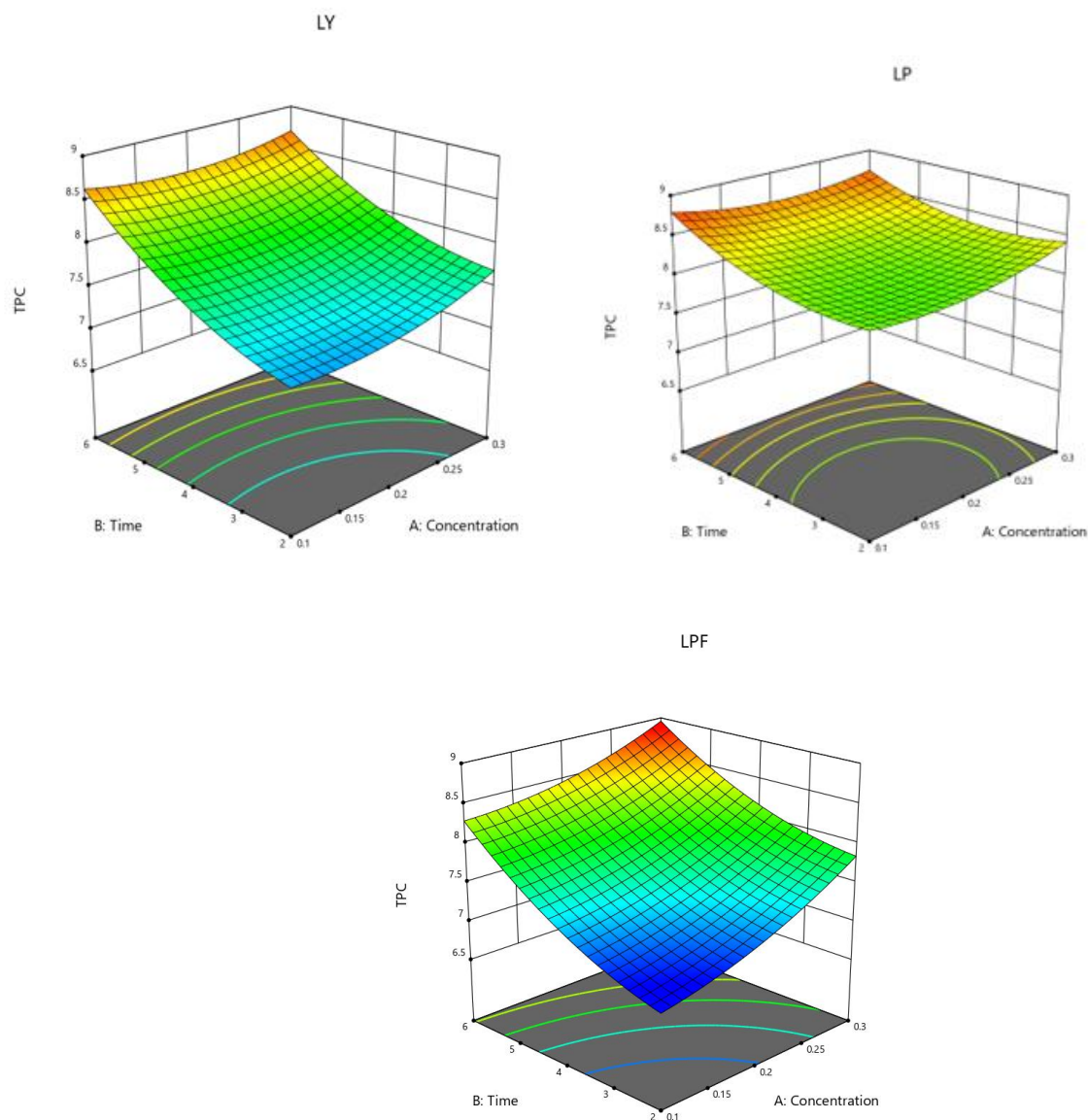


Fig 4.6 Effect of process parameters on TPC

The ANOVA results indicated that fermentation time (Factor B) exerted the greatest influence on the total plate count of fermented idli batter, as evidenced by the highest F-value (51.91) and a highly significant p-value (<0.0001). This finding demonstrates that fermentation duration was the primary factor governing microbial growth. As fermentation progressed, favourable conditions promoted the multiplication of lactic acid bacteria, resulting in a significant increase in viable microbial counts. The increase in microbial population is attributed to enhanced metabolic activity and utilization of available nutrients during fermentation.

The starter culture concentration (Factor A) also had a significant effect on total plate count ($F = 8.34$; $p = 0.0075$), indicating that increasing the inoculum concentration enhanced microbial proliferation. Similarly, the type of starter culture (Factor C) significantly influenced microbial growth ($F = 13.29$; $p < 0.0001$), suggesting that the microbial combinations differed in their ability to grow and establish within the idli batter. These findings indicate that both inoculum concentration and starter culture composition play important roles in determining the viable microbial population during fermentation.

Among the interaction terms, the interaction between fermentation time and starter culture type (BC) was statistically significant ($F = 3.55$; $p = 0.0428$), indicating that the effect of fermentation time on microbial growth depended on the type of starter culture employed. However, the interaction between starter culture concentration and fermentation time (AB) ($p = 0.3211$) and between starter culture concentration and starter culture type (AC) ($p = 0.0781$) were not statistically significant, suggesting that these combinations had minimal influence on the total plate count.

Regarding the quadratic effects, B^2 showed a significant effect ($F = 4.22$; $p = 0.0498$), indicating a slight non-linear relationship between fermentation time and microbial population. In contrast, the quadratic effect of starter culture concentration (A^2) was not significant ($p = 0.1637$), suggesting that microbial growth responded approximately linearly to changes in inoculum concentration within the investigated range.

The non-significant lack-of-fit ($F = 2.16$; $p = 0.0930$) confirmed that the developed regression model adequately represented the experimental data and could reliably predict the total plate count under the selected processing conditions. Overall, the statistical analysis demonstrated that fermentation time was the most influential factor affecting total plate count, followed by starter culture type and starter culture concentration. The significant BC interaction further indicated that microbial growth was influenced by the combined effect of fermentation time and starter culture type. These findings highlight the importance of optimizing fermentation time together with appropriate starter culture selection to achieve desirable microbial growth, improved fermentation efficiency, and enhanced microbiological quality of fermented idli batter.

4.3.2 Effect of Process Parameters on Sensory Evaluation of Fermented Idli

Sensory evaluation is an important measure of the overall quality and consumer acceptability of fermented idli. Although physico-chemical and microbiological analyses assess fermentation performance, the final product is judged by its appearance, aroma, taste, texture, softness, sponginess, and overall acceptability.

Table 4.14. Sensory Evaluation Scores of Fermented Idli

SI NO.	COMBINATION	CONCENTRATION	TIME	SENSORY SCORE
1	Control			6.8
2	LY	0.1	2	6.4
3	LPF	0.2	2	7.1
4	LP	0.2	2	7.8
5	LY	0.3	2	6.2
6	LP	0.3	4	7.5
7	LPF	0.1	4	8.1
8	LPF	0.3	4	8
9	LY	0.2	4	6.2
10	LP	0.1	4	7.6
11	LPF	0.3	6	8.8
12	LY	0.3	6	6
13	LP	0.2	6	8.4
14	LY	0.1	6	6.1

The results showed that starter culture combination, inoculum concentration, and fermentation time significantly influenced the sensory quality of idli. The control sample, which underwent spontaneous fermentation, recorded an overall sensory score of 6.8, indicating moderate acceptability. In contrast, all starter culture-treated samples exhibited improved sensory characteristics. Among the treatments, the LPF (*Lactococcus lactis* + *Lactobacillus plantarum* +

Lactobacillus fermentum) at 0.3 ppm fermented for 6 h achieved the highest overall acceptability score (8.8). The idlis prepared from this treatment exhibited an attractive appearance, pleasant aroma, mild sour taste, soft and spongy texture, and excellent mouthfeel. The LP treatment at 0.2 ppm for 6 h also showed high acceptability (8.4), whereas the LY treatment recorded a comparatively lower score (6.0), indicating that excessive acidification and gas production adversely affected flavour and texture despite higher batter expansion. Sensory quality closely corresponded with the physico-chemical characteristics of the batter. Treatments with higher volume rise, lower bulk density, and moderate acidification produced softer, more porous idlis with superior flavour and texture. The balanced fermentation achieved by the LPF formulation resulted in improved batter aeration, controlled acid production, and enhanced overall product quality.

The findings of the present investigation are consistent with previous studies on controlled fermentation of cereal-legume products. Rao *et al.*, (2023) reported that mixed starter cultures significantly improved the texture, flavour, and overall acceptability of fermented cereal products through balanced fermentation. Similarly, Martínez *et al.*, (2024) demonstrated that defined lactic acid bacterial starter cultures enhanced sensory quality by promoting controlled acidification and desirable flavour compound production. The LPF treatment achieved an overall sensory score of 8.8, representing approximately a 29% improvement over the control (6.8), and slightly exceeding the acceptability scores (8.10–8.38) reported for other defined starter culture combinations.

Overall, the sensory evaluation validated the optimization strategy adopted in the present study. The treatment identified as optimum through physico-chemical and microbiological analyses also achieved the highest sensory acceptability, demonstrating strong agreement between instrumental measurements and consumer perception. Among all treatments, the LPF combination at 0.3 ppm fermented for 6 h produced the most desirable balance of appearance, flavour, aroma, texture, softness, and sponginess, confirming its potential for the production of standardized idli batter with superior consumer acceptance and commercial applicability.

The effect of process parameters on the sensory quality of fermented idli was evaluated using analysis of variance (ANOVA), and the results are presented in Table 4.14. The ANOVA results revealed that the regression model was highly significant ($F = 12.63$; $p < 0.0001$), indicating that the selected model adequately explained the variation in sensory scores obtained under

different fermentation conditions. Furthermore, the non-significant lack-of-fit ($p = 0.1000$) confirmed that the regression model provided an adequate fit to the experimental data and could reliably predict the sensory response within the selected experimental range.

Table 4.15. Analysis of variance (ANOVA) for sensory evaluation

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	26.68	11	2.43	12.63	< 0.0001	significant
A- Concentration	2.49	1	2.49	12.99	0.0013	
B-Time	1.45	1	1.45	7.52	0.0107	
C-Organism	20.75	2	10.38	54.03	< 0.0001	
AB	0.27	1	0.27	1.41	0.2461	
AC	0.1811	2	0.0906	0.4715	0.6291	
BC	0.4433	2	0.2217	1.15	0.3304	
A ²	0.9819	1	0.9819	5.11	0.032	
B ²	0.0042	1	0.0042	0.0217	0.8841	
Residual	5.19	27	0.1921			
Lack of Fit	3.76	15	0.2505	2.11	0.1	not significant
Pure Error	1.43	12	0.119			
Cor Total	31.87	38				

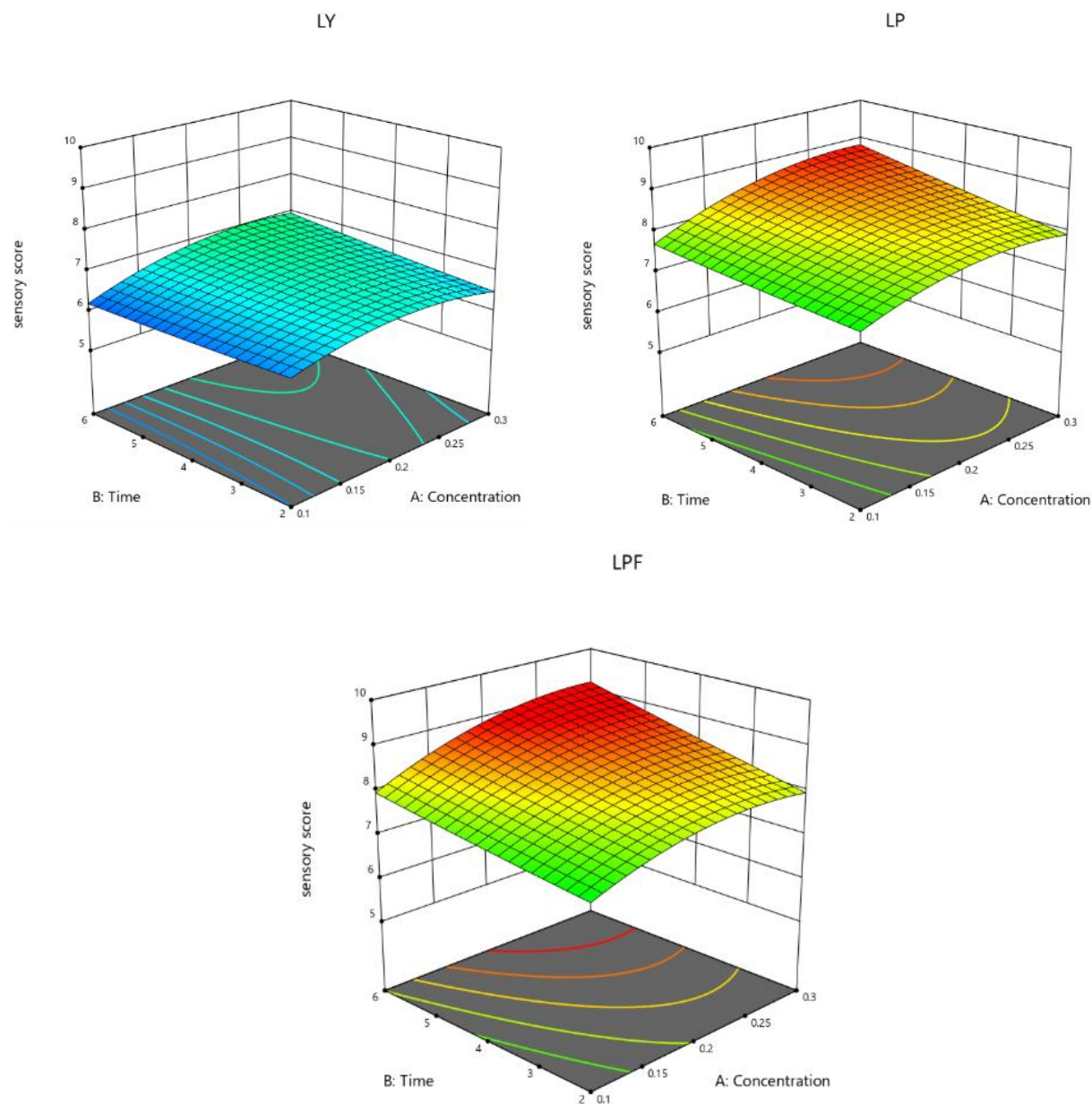


Fig 4.7: Effect of process parameters on sensory analysis

The ANOVA results indicated that the type of starter culture (Factor C) had the greatest influence on the sensory quality of fermented idli, as evidenced by the highest F-value (54.03) and a highly significant p-value (<0.0001). This finding demonstrates that the selection of an appropriate starter culture plays a crucial role in determining the overall sensory acceptability of the product. Differences in microbial composition influenced flavour development, aroma, texture, and appearance, thereby significantly affecting panel preference.

The starter culture concentration (Factor A) also significantly influenced sensory evaluation ($F = 12.99$; $p = 0.0013$). Increasing the inoculum concentration improved fermentation efficiency, resulting in better flavour development, softer texture, enhanced sponginess, and higher overall acceptability. Similarly, fermentation time (Factor B) had a significant effect ($F = 7.52$; $p = 0.0107$), indicating that sufficient fermentation time was necessary to achieve desirable sensory characteristics. Appropriate fermentation promoted balanced acid production and carbon dioxide formation, leading to improved texture and flavour, whereas inadequate or excessive fermentation could adversely affect product quality.

The interaction effects between starter culture concentration and fermentation time (AB), starter culture concentration and organism (AC), and fermentation time and organism (BC) were not statistically significant ($p > 0.05$), indicating that the combined influence of these factors did not significantly affect the sensory attributes of fermented idli. Thus, the main effects of concentration, fermentation time, and starter culture type were more influential than their interactions.

Among the quadratic terms, A^2 was statistically significant ($F = 5.11$; $p = 0.0320$), suggesting a slight non-linear relationship between starter culture concentration and sensory score. However, the quadratic effect of fermentation time (B^2) was not significant ($p = 0.8841$), indicating that sensory quality varied almost linearly within the investigated fermentation period.

The non-significant lack-of-fit ($F = 2.11$; $p = 0.1000$) confirmed that the regression model adequately represented the experimental data and could reliably predict sensory responses under the selected processing conditions. Overall, the statistical analysis demonstrated that starter culture type was the most influential factor affecting sensory quality, followed by starter culture concentration and fermentation time, whereas the interaction effects showed no significant influence. These findings indicate that selecting an appropriate starter culture and optimizing its concentration and fermentation time are essential for producing fermented idli with superior sensory attributes and enhanced consumer acceptability.

4.4 OPTIMIZATION OF STARTER CULTURE FOR IDLI BATTER FERMENTATION

Optimization of the starter culture was carried out to identify the most suitable combination of lactic acid bacteria, inoculum concentration, and fermentation time for the production of high-

quality fermented idli batter. The optimization process was based on the combined evaluation of physico-chemical properties, microbiological quality, and sensory characteristics obtained during the present investigation. Response Surface Methodology (RSM) and Design-Expert software were employed to evaluate the individual and interactive effects of the selected process variables and to determine the optimum fermentation conditions.

Based on the experimental design, three starter culture combinations, namely LY, LP, and LPF, were evaluated at different concentrations and fermentation periods. Initially, 27 experimental combinations were generated, which were reduced to 17 using Design-Expert® software by eliminating statistically redundant combinations. The experimental design was further refined to obtain 14 unique treatment combinations for detailed analysis.

The independent variables considered for optimization were starter culture combination, starter culture concentration, and fermentation time, while the dependent variables included L* value, volume rise, pH, bulk density, total plate count (TPC), titratable acidity, and sensory score. The 14 experimental treatments showed considerable variation in the measured responses depending on the starter culture combination, concentration, and fermentation time. In general, prolonged fermentation resulted in increased volume rise, microbial count, and titratable acidity, along with a reduction in pH and bulk density. The sensory scores also varied among the treatments, indicating the influence of starter culture and fermentation conditions on the overall quality of idli batter.

For optimization, a multi-response desirability approach was adopted by assigning suitable desirability directions to each quality attribute. L* value, volume rise, microbial count, and sensory score were maximized, whereas bulk density was minimized. Titratable acidity and pH were assigned target values to achieve balanced fermentation and prevent excessive acid development. The desired region for titratable acidity was approximately 0.5–0.8% lactic acid, while the target pH range was approximately 4.5–5.0. The optimization criteria used for the selection of the desirable starter culture treatment are presented in Table 4.15.

Table 4.16. Optimization criteria used for the selection of starter culture and fermentation conditions

Parameter	Desirability direction	Low range	High range	Optimum/desired region
Bulk density (g/mL)	Minimize	0.80	1.00	Lower value
L* value	Maximize	50	80	Higher value
Volume rise (mL)	Maximize	20	100	Higher value
Microbial count (log CFU/g)	Maximize	5	9	Higher value
Sensory score (9-point scale)	Maximize	5	9	Higher value
Titrateable acidity (% lactic acid)	Target	0.3	1.0	Approximately 0.5–0.8
pH	Target	4.0	6.0	Approximately 4.5–5.0

Among the 14 experimental treatments, LPF at 0.3 concentration fermented for 6 h (Run 11) and LP at 0.2 concentration fermented for 6 h (Run 13) exhibited the most desirable overall response characteristics. The LPF 0.3 treatment recorded an L* value of 78.4, volume rise of 38 mL, pH of 4.8, bulk density of 0.72 g/mL, TPC of 8.7 log CFU/g, titrateable acidity of 0.68% lactic acid, and sensory score of 8.8. Similarly, LP 0.2 recorded an L* value of 78.7, volume rise of 34 mL, pH of 4.9, bulk density of 0.75 g/mL, TPC of 8.5 log CFU/g, titrateable acidity of 0.65% lactic acid, and sensory score of 8.4.

The LPF 0.3 treatment showed the highest sensory score (8.8) and the greatest volume rise (38 mL) among the selected treatments, together with a desirable pH of 4.8, titrateable acidity of 0.68% lactic acid, and lower bulk density of 0.72 g/mL. These characteristics indicated effective fermentation, improved batter aeration, and good overall product acceptability. The LP 0.2

treatment also exhibited a favourable quality profile, with a slightly higher L* value of 78.7, pH of 4.9, titratable acidity of 0.65% lactic acid, and sensory score of 8.4. The corresponding experimental results confirmed the superior performance of these two treatments compared with the other starter culture combinations and concentrations.

Table 4.17. Response characteristics of the selected optimized starter culture treatments

Parameter	LPF 0.3, 6 h	LP 0.2, 6 h
L* value	78.4	78.7
Volume rise (mL)	38	34
pH	4.8	4.9
Bulk density (g/mL)	0.72	0.75
TPC (log CFU/g)	8.7	8.5
Titratable acidity (% lactic acid)	0.68	0.65
Sensory score	8.8	8.4

The selection of the optimized treatments was based on the combined performance of all the evaluated responses rather than on a single quality parameter. The LPF 0.3 and LP 0.2 treatments fermented for 6 h showed desirable colour, volume development, acidity, pH, bulk density, microbial activity, and sensory characteristics. Sensory evaluation further demonstrated that these two treatments produced idlis with better appearance, texture, flavour, and overall acceptability compared with the other experimental treatments. Therefore, LPF 0.3 and LP 0.2 fermented for 6 h were selected as the optimized treatments for further storage studies.

4.5. SHELF-LIFE STUDY OF FERMENTED IDLY BATTER

The shelf life study of the optimized fermented idli batter was carried out to evaluate the stability and quality retention of the product during refrigerated storage. Based on the optimization study, LPF at a concentration of 0.3 mL/100 g batter, comprising *Lactococcus lactis*, *Lactobacillus plantarum*, and *Lactobacillus fermentum*, was identified as the standardized starter culture

treatment. LP at 0.2 mL/100 g batter was also retained as an optimized treatment for comparison. The optimized fermented batter was packed in 80 µm laminated pouches and stored under refrigerated conditions. Samples were evaluated at 0th, 7th, 14th, and 21st days of storage to monitor changes in physico-chemical, microbiological, and sensory characteristics. The storage study was conducted to determine the period up to which the fermented idli batter retained desirable quality and consumer acceptability.

During refrigerated storage, the fermented batter exhibited gradual changes in its physico-chemical characteristics, which could be attributed to the continued metabolic activity of the starter microorganisms. Although refrigeration reduced the rate of fermentation, the microorganisms remained metabolically active and continued to produce organic acids and other fermentation metabolites. Consequently, changes were observed in colour, volume rise, pH, bulk density, and titratable acidity throughout the storage period. These changes were more pronounced with prolonged storage and were particularly evident by the 21st day.

The colour of the fermented batter remained relatively stable during the initial stages of storage. For the LPF 0.3 treatment, the L* value was 81.80 on the 0th day, 82.35 on the 7th day, and 82.50 on the 14th day, indicating that the batter retained its desirable light colour during the recommended storage period. The limited variation in L* value suggested that refrigerated storage did not cause substantial deterioration in the visual appearance of the batter.

A progressive increase in volume rise was observed during storage. In the LPF 0.3 treatment, volume rise increased from 1.85 on the 0th day to 2.50 on the 7th day and 2.90 on the 14th day. The increase in volume development indicated that the starter microorganisms remained active during refrigerated storage and continued to contribute to fermentation and gas production. The relatively higher volume rise observed for LPF 0.3 suggested improved fermentation performance and batter aeration compared with the other treatment.

The pH of the fermented batter showed a progressive decline with increasing storage duration. In the LPF 0.3 treatment, pH decreased from 6.1 on the 0th day to 4.8 on the 7th day, 4.4 on the 14th day, and 3.5 on the 21st day. The reduction in pH was indicative of continued acid production by the starter microorganisms during refrigerated storage. The corresponding increase in titratable acidity further confirmed the progression of fermentation.

Similarly, bulk density decreased progressively during storage. In LPF 0.3, bulk density decreased from 0.87 g/mL on the 0th day to 0.72 g/mL on the 7th day and 0.63 g/mL on the 14th day. The decrease in bulk density may be associated with continued fermentation and gas production, resulting in increased aeration and expansion of the batter. This change was favourable from the standpoint of idli quality, as a less dense batter can contribute to the development of a softer and more porous product.

Titrateable acidity increased progressively during storage, corresponding to the reduction in pH. In LPF 0.3, titrateable acidity increased from 0.68% lactic acid on the 0th day to 1.32% on the 7th day and 1.48% on the 14th day. The increase indicated continued production of organic acids by the starter microorganisms under refrigerated conditions. While moderate acid development is an important characteristic of fermented idli batter, excessive acid accumulation during prolonged storage can result in excessive sourness and adversely affect sensory quality.

The microbial characteristics of the optimized batter also reflected the continued activity of the starter culture during storage. The LPF 0.3 treatment showed a TPC of 8.7 log CFU/g under the optimized fermentation condition, indicating active microbial growth and fermentation. The microbial activity was associated with the observed changes in pH, titrateable acidity, volume rise, and bulk density. However, microbial characteristics were considered together with physico-chemical and sensory responses for determining the practical shelf life of the product.

Sensory acceptability was considered the major criterion for determining the recommended shelf life of the fermented idli batter. The sensory characteristics showed a gradual decline with increasing storage duration. For the LPF 0.3 treatment, the overall acceptability score was 8.5 on the 0th day, which decreased to 7.33 on the 7th day and 7.0 on the 14th day. By the 21st day, the overall acceptability further decreased to 6.33. The decline in sensory quality during prolonged storage was associated with changes in flavour, taste, mouthfeel, and aftertaste, which can be related to the continued fermentation and accumulation of organic acids during storage.

Among the optimized treatments, LPF 0.3 mL/100 g batter showed comparatively better storage performance and sensory acceptability. The treatment maintained desirable colour, volume development, batter structure, and sensory characteristics during the storage period. The higher sensory acceptability observed for LPF 0.3 may be attributed to the balanced fermentation activity provided by the combination of *L. lactis*, *L. plantarum*, and *L. fermentum*. The results therefore

indicated that the LPF starter culture was effective in maintaining the quality characteristics of fermented idli batter during refrigerated storage. Although the samples were evaluated up to the 21st day, the progressive reduction in sensory acceptability together with the marked changes in pH and titratable acidity indicated that prolonged storage was not desirable. The 14th day was therefore considered the recommended shelf life of the fermented idli batter under the storage conditions employed in the present study. The recommendation was primarily based on sensory acceptability and was supported by the observed physico-chemical and microbiological changes. At the 14th day, the batter still retained acceptable sensory characteristics, whereas further storage resulted in a noticeable decline in overall acceptability.

Thus, the study established that the optimized LPF starter culture at 0.3 mL/100 g batter with 6 h fermentation, when packed in 80 µm laminated pouches and stored under refrigerated conditions, could maintain desirable quality characteristics for up to 14 days. Although quality evaluation was extended to 21 days, storage beyond 14 days was not recommended because of the decline in sensory acceptability and the progressive changes associated with continued fermentation. Therefore, 14 days was established as the recommended refrigerated shelf life of the optimized fermented idli batter, demonstrating the potential of selected starter cultures for producing a stable and acceptable fermented idli batter with extended storage life.

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

Idli is a popular traditional fermented food widely consumed in South India and is valued for its soft and spongy texture, pleasant taste, nutritional quality, and easy digestibility. Traditionally, idli batter undergoes natural fermentation through the activity of microorganisms naturally present in the raw materials and the surrounding environment. However, variations in the microbial population and fermentation conditions can result in batch to batch differences in texture, flavour, volume, acidity, and storage stability. Therefore, the present study was undertaken to standardize the fermentation of idli batter using selected starter cultures with the objective of obtaining a more consistent fermentation, with desirable quality characteristics and improved storage stability.

The study was initiated with the selection and screening of five potential starter cultures, namely *Lactobacillus delbrueckii* subsp. *Bulgaricus*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactococcus lactis*, and *Saccharomyces cerevisiae*. The cultures were initially evaluated individually to determine its suitability for idli batter fermentation based on their fermentation performance and its effect on the quality characteristics of the final product. Based on the screening results, suitable cultures were selected and used to develop different starter culture combinations, namely LY, LP, and LPF, for mixed-culture fermentation of idli batter.

The fermentation process was optimized using Response Surface Methodology (RSM) with Design-Expert software. Initially, 27 experimental combinations were generated, which were reduced to 17 experimental runs and subsequently refined to 14 unique treatment combinations for detailed evaluation. Starter culture combination, inoculum concentration, and fermentation time were considered as the independent variables, while L* value, volume rise, pH, bulk density, total plate count, titratable acidity, and sensory score were evaluated as responses. Based on the experimental design, the resulting treatment combinations were evaluated at fermentation periods of 2, 4, and 6 h to determine the influence of fermentation time and inoculum concentration on the physicochemical, microbiological, and sensory characteristics of idli batter. The optimized treatments obtained from the fermentation study were subsequently selected for further evaluation under refrigerated storage conditions.

A refrigerated storage study was subsequently conducted to assess the stability and acceptability of the optimized idli batter during storage. The fermented batter was packed in 80 μ m laminated pouches and stored under refrigerated conditions. The quality of the batter was evaluated at regular intervals during storage by monitoring selected physicochemical, microbiological, and sensory characteristics.

The results of the above experiments are summarized as follows:

The preliminary screening of the selected starter cultures showed clear differences in their fermentation performance and sensory characteristics. Among the five cultures evaluated, *Lactobacillus delbrueckii* subsp. *Bulgaricus* showed comparatively lower fermentation efficiency and sensory acceptability. Hence, it was excluded from further optimization studies. The remaining cultures, namely *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactococcus lactis*, and *Saccharomyces cerevisiae*, were selected for further investigation and used to develop the LY, LP, and LPF starter cultures.

The experimental design initially generated 27 combinations, which were reduced to 17 experimental runs and subsequently refined to 14 unique treatment combinations for detailed evaluation. The treatment combinations were studied at fermentation periods of 2, 4, and 6 h and at different inoculum concentrations. The responses obtained from the physicochemical, microbiological, and sensory analyses demonstrated progressive changes with fermentation time. Based on the combined responses, 6 h was identified as the optimum fermentation period. Under the optimized conditions, LPF at an inoculum concentration of 0.3 mL and LP at 0.2 mL per 100 g batter (LPF 0.3 and LP 0.2, respectively) were identified as the optimized formulations and selected for further evaluation.

Among the optimized formulations, LPF 0.3 showed the more desirable overall response, with an L* value of 78.4, volume rise of 38 mL, pH of 4.8, bulk density of 0.72 g/mL, total plate count of 8.7 log CFU/g, titratable acidity of 0.68 per cent lactic acid, and sensory score of 8.8. The corresponding values for LP 0.2 were 78.7, 34 mL, 4.9, 0.75 g/mL, 8.5 log CFU/g, 0.65 per cent lactic acid, and 8.4, respectively. These findings indicated that both optimized treatments produced desirable fermentation characteristics, with LPF 0.3 showing comparatively better overall performance. The observed increase in volume rise and titratable acidity, along with the reduction in pH and bulk density, indicated effective fermentation and development of desirable batter

characteristics. The microbial population remained within acceptable limits, supporting the microbiological quality of the optimized treatments.

Sensory evaluation using a nine-point hedonic scale showed that the optimized LP 0.2 and LPF 0.3 formulation received higher scores for colour, appearance, aroma, texture, taste, softness, and overall acceptability compared with the other formulations. The selected starter culture combination contributed to the development of idlis with improved texture, enhanced flavour, and better overall consumer acceptance.

The optimized treatments were subsequently subjected to refrigerated storage to assess their stability and acceptability during storage. The quality of the batter was evaluated on the 0th, 7th, 14th and 21st days of storage. During the storage period, a gradual decrease in pH along with an increase in titratable acidity was observed, indicating the continuation of fermentation under refrigerated conditions. Changes in volume rise and bulk density were also observed as storage progressed. Compared with the control, the starter culture-treated samples showed better control of acidification and higher sensory acceptability. Among the optimized treatments, LPF 0.3 mL/100 g batter showed better overall performance during refrigerated storage. The overall sensory score of LPF 0.3 decreased from 8.5 on day 0 to 7.33, 7.0 and 6.33 on days 7, 14 and 21, respectively. Although the batter remained acceptable during the earlier stages of storage, the progressive acidification and decline in sensory acceptability at 21 days indicated a loss of desirable quality. Therefore, 14 days was considered the acceptable refrigerated storage period for the optimized batter under the conditions of the present study.

Following conclusions were derived based on the findings:

- The study demonstrated that the use of selected starter cultures could be used to control and standardize the fermentation of idli batter, resulting in improved and consistent product quality.
- Among the five starter cultures evaluated, the combination of *Lactococcus lactis*, *Lactobacillus plantarum*, and *Lactobacillus fermentum* (LP and LPF respectively) was identified as the most suitable culture combination. It produced idlis with desirable physicochemical, microbiological, and sensory characteristics.
- Response Surface Methodology using Design-Expert software successfully identified the optimum fermentation conditions. Among the fermentation periods evaluated, 6 h was

identified as the optimum fermentation period, and LPF 0.3 and LP 0.2 mL per 100 g batter were selected as the optimized formulations.

- The optimized fermentation conditions, consisting of an inoculum concentration of 0.3 mL and 0.2 mL per 100 g of batter for LPF and LP, respectively along with a fermentation period of 6 hours, resulted in idlis with improved texture, volume, softness, flavour, and overall consumer acceptability compared with conventionally fermented samples.
- Controlled fermentation using the selected starter culture helped maintain desirable physicochemical characteristics of the batter, including pH, titratable acidity, colour, and bulk density, while also supporting microbiological safety.
- During refrigerated storage, the optimized treatments showed gradual changes in physicochemical and sensory characteristics. LPF 0.3 mL/100 g batter retained satisfactory overall quality for up to 14 days under refrigerated storage, whereas the decline in sensory acceptability and progressive acidification at 21 days indicated that further storage was undesirable.
- The optimized idli batter remained microbiologically safe and organoleptically acceptable for up to 14 days under refrigerated storage indicating a considerable improvement in its storage stability compared with traditionally fermented batter.
- Overall, the application of selected starter cultures provided a more reliable and reproducible fermentation process, with reduced batch-to-batch variation and improved consistency in the quality of the final product.

Scope of future work

- Improving the shelf life of idli batter through the use of suitable packaging materials and packaging technologies.
- Evaluating the performance of the selected starter culture under large-scale and commercial production conditions.
- Incorporating nutritionally beneficial ingredients such as millets and pulses to enhance the nutritional value of idli.
- Studying the beneficial microbial cultures in greater detail, including their contribution to fermentation, flavour development, and product quality.

- Conducting consumer acceptance studies with a larger and more diverse group of consumers.
- Evaluating the applicability of the selected starter culture for the fermentation of other traditional fermented food products.
- Optimizing the fermentation process for industrial-scale production while maintaining product quality and consistency.
- Carrying out further studies to improve the overall quality, stability, nutritional value, and consumer acceptability of the product.

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CHAPTER VI

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APPENDIX

APPENDIX A

SENSORY EVALUATION CARD

SENSORY EVALUATION (9. HEDONIC SCALE)

IDL

ATTRIBUTES	SAMPLE CODE	SAMPLE CODE	SAMPLE CODE	SAMPLE CODE
Color				
Appearance				
Texture (Sponginess, Fluffiness, Firmness, Stickiness)				
Flavour/Aroma				
Taste				
Mouthfeel (Sourness)				
After Taste				
Overall Acceptability				

9-Like Extremely
8-Like very much
7-Like
6-Like slightly
5-Neither like or dislike
4-Dislike slightly
3-Dislike moderately
2-Dislike
1-Dislike extremely

NAME:

DESIGNATION:

SIGNATURE:

APPENDIX B

COMPOSITION OF CULTURE MEDIA

B.1 Plate count agar

Ingredients	g/L
Enzymatic digestion of casein	5.0 g
Yeast extract	2.5 g
Glucose anhydrous	1.0 g
Agar	9 g to 18 g

B.2 MRS Agar

Ingredients	g/L
Meat extract	8.0 g
Peptone	10.0 g
Yeast extract	5.0 g
Tri-ammonium citrate	2.0 g
Sodium acetate	5.0 g
Magnesium sulphate, heptahydrate	0.20 g
Manganese sulphate, tetrahydrate	0.050 g
Dipotassium Hydrogen phosphate	2.0 g
Glucose	20 g
Sorbitan monooleate (Tween 80)	1.0 g
Agar	12 g to 18 g

B.3 Nutrient agar

Ingredients	g/L
Yeast extract	1.5 g
Peptone	5.0 g
Agar	15.0 g
Sodium chloride	5.0 g

HM peptone B	1.5 g
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B.4 Nutrient broth

Ingredients	g/L
Peptone	5.00 g
Sodium chloride	5.00 g
Yeast extract	1.5 g
HM peptone B	1.5 g

**STANDARDIZATION OF PROCESS PARAMETERS FOR IDLI BATTER
FERMENTATION USING SELECTED STARTER CULTURE
COMBINATIONS**

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ABSTRACT OF PROJECT REPORT

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DEPARTMENT OF PROCESSING AND FOOD ENGINEERING

**KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND FOOD
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STANDARDIZATION OF PROCESS PARAMETERS FOR IDLI BATTER FERMENTATION USING SELECTED STARTER CULTURE COMBINATIONS

ABSTRACT

The present study was carried out to standardize the fermentation of idli batter using selected starter culture combinations, with the aim of achieving consistent fermentation and extending its refrigerated shelf life. To achieve this, five starter cultures, namely *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactococcus lactis*, and *Saccharomyces cerevisiae*, were selected based on their acidification ability, carbon dioxide production, enzymatic activity, and compatibility during mixed-culture fermentation. The selected starter cultures were evaluated in different combinations and inoculum concentrations, and Response Surface Methodology (RSM) using Design-Expert software was employed to optimize the starter culture combination, inoculum concentration, and fermentation time. The resulting idli batters were then evaluated for changes in physico-chemical properties, sensory characteristics, and microbiological quality at fermentation periods of 2, 4 and 6 hours. Based on the responses obtained at different fermentation periods and inoculum concentrations, 6 h was identified as optimum. Among the optimized formulations, LPF at inoculum concentrations of 0.3 mL and LP at 0.2 mL per 100 g batter (LPF 0.3 and LP 0.2 respectively) were selected for further evaluation.

The optimized treatments were subsequently subjected to a refrigerated storage study. The fermented batter was packed in 80 µm laminated pouches and stored under refrigerated conditions. Quality evaluation was carried out on the 0th, 7th, 14th and 21st days of storage. Among the optimized treatments, LPF 0.3 mL/100 g batter showed the most desirable overall response. A gradual decrease in pH along with an increase in titratable acidity was observed, indicating the progress of controlled fermentation. The production of carbon dioxide by the starter cultures contributed to a reduction in bulk density and an increase in volume expansion of the batter, resulting in idlis with a soft, porous, and spongy texture. Compared with the control, the starter culture-treated samples showed better colour stability, controlled acidification, and higher sensory acceptability during storage. Among the different treatments, the optimized LPF consortium, applied as a centrifuged cell suspension at 0.3 ml/100g batter,

retained satisfactory physico-chemical, microbiological and sensory quality for up to 14 days under refrigerated storage. The study indicates that the use of a suitable starter culture combination can help achieve controlled and consistent fermentation of idli batter while improving its quality and refrigerated shelf life.

Keywords: Starter culture consortium; Controlled fermentation; Lactic acid bacteria; Shelf-life extension; Refrigerated storage.

