Optimization and characterization of functional instant *Idli* mix

THESIS SUBMITTED TO THE

ICAR-NATIONAL DAIRY RESEARCH INSTITUTE

(DEEMED UNIVERSITY)

IN PARTIAL FULLFILMENT OF THE REQUIREMENTS FOR

THE DEGREE OF

MASTER OF SCIENCE

IN

FOOD TECHNOLOGY

BY

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B.Sc (Food Nutrition and Dietetics)

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ICAR-NATIONAL DAIRY RESEARCH INSTITUTE

KARNAL-132001 (HARYANA), INDIA

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दिनांक /**Dated: 25.09.2021**

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CERTIFICATE

This is to certify that the thesis entitled **'Optimization and characterization of functional instant *idli* mix'** submitted by **Ms Eswari E** towards the partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE** in **FOOD TECHNOLOGY** of the **ICAR-NATIONAL DAIRY RESEARCH INSTITUTE (DEEMED UNIVERSITY)**, Karnal (Haryana), India, is a bonafide research work carried out by her under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

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DEDICATED TO MY BELOVED FAMILY, FRIENDS, GUIDE AND GOD

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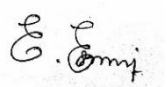
There are still so many people who have knowingly and unknowingly helped me, whose name might not appear in the acknowledgement but the sense of gratitude for them will always remain in my heart. I express my heartfelt gratitude to each and every one of them.



ESWARI E

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Optimization and Characterization of Functional Instant *Idli* mix

Scholar	Major Advisor	Division
ESWARI E	DR. NEELAM UPADHYAY	DAIRY TECHNOLOGY

ABSTRACT

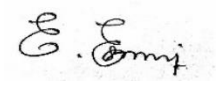
Emerging research and technologies aim to satisfy customers by exploring new food products. An instant food preparation like ready to cook and ready to serve, etc have found a vital place in the modern era. This is because instant food at household level needs lesser preparation time and minimum handling. At the same time, consumer demands to have healthier ingredients possessing functional attributes in instant foods. Considering these requirements, the present study was planned to incorporate functional ingredients into instant *idli* mix for improving its functional properties without causing detrimental effect on the organoleptic properties of *idli*. Initially, descriptive sensory evaluation, texture profile analysis and ink print tests were done for 10 different market *idlies* to fix range of all parameters for optimizing functional multi-grain instant *idli* mix (FMIIDM). One-way ANOVA was applied on all the parameters of market sample of *idli*. The results indicated significant ($p<0.05$) difference on firmness, fermented aroma, acidic taste, all texture profile analysis parameters and ink print test. The proximate analysis was done for raw rice, urad dhal, sorghum and *Moringa* pod powder (MPP). MPP showed significantly higher ($P<0.05$) level of fat (15.5±0.55%) and ash (6.37±0.07%) over the other ingredients used. The processing steps and ingredients were optimized for developing FMIIDM. The optimization was done using 2^5 factorial experiment on SPSS. The organoleptic properties, texture profile and ink print test for 32 combinations was carried out to select the optimized combination. Based on the optimized level of variables, two controls were prepared following optimized steps of preparation of functional instant *idli*. The proximate composition of FMIIDM showed significantly ($p<0.05$) high protein, fat, ash and moisture content than controls. Colour and a_w of FMIDM showed significant ($p<0.05$) difference over controls. The bio-functional attributes i.e., total carotenoids, phenolics, flavonoids, vitamin C, iron, calcium and anti-oxidant activities showed significantly ($p<0.05$) higher values than controls. The descriptive sensory analysis showed significant ($p<0.05$) difference with respect to color as well as dryness interior of *idli* prepared from FMIIDM than controls while other parameters showed non-significant ($p>0.05$) difference with respect to controls. All parameters of texture profile except hardness showed significant ($p<0.05$) difference between controls and FMIIDM. The current research study concludes that addition of optimized product offered excellent bio-functional qualities without detrimental effect on organoleptic properties and textural profile of the *idli* prepared.

Scholar **Major Advisor**

कार्यात्मक त्वरित इडली मिश्रण का इष्टमीकरण और विशेलेष्ण

सार

अनुसंधान और प्रौद्योगिकियों के विकास का उद्देश्य नए खाद्य उत्पादों की खोज कर ग्राहकों को संतुष्ट करना है। तत्काल भोजन की तैयारी जैसे पकाना, तैयार करना और परोसने आदि ने आधुनिक युग में एक महत्वपूर्ण स्थान प्राप्त किया है। ऐसा इसलिए है क्योंकि घरेलू स्तर पर त्वरित भोजन तैयार करने में कम समय लगता है और इसे कम संभालना पडता है । साथ ही, उपभोक्ता इन्स्टेन्ट (त्वरित) खाद्य पदार्थों में कार्यात्मक गुणों वाले स्वस्थ अवयवों की मांग करते हैं। वर्तमान अध्ययन की योजना इडली के संवदिक गुणों पर हानिकारक प्रभाव डाले बिना इसके कार्यात्मक गुणों में सुधार के लिए तत्काल इडली मिश्रण में कार्यात्मक अवयवों को शामिल करने की आवश्यकताओं को ध्यान में रखते हुए बनाई गई थी। प्रारंभ में, कार्यात्मक मल्टी-ग्रेन इंस्टेंट इडली मिक्स (क.म.ग्र.इं.इ.म.) के अनुकूलन के लिए सभी मापदंडों की सीमा तय करने के लिए 10 अलग-अलग मार्केट इडली के लिए वर्णनात्मक संवेदी मूल्यांकन, बनावट प्रोफ़ाइल विश्लेषण और स्याही प्रिंट परीक्षण किए गए थे। इडली के मार्केट सैंपल के सभी मापदंडों पर वन-वे एनोवा लागू किया गया था। परिणाम ने ठोसपन, किण्वित सुगंध, अम्लीय स्वाद, सभी बनावट प्रोफ़ाइल विश्लेषण मापदंडों और स्याही प्रिंट परीक्षण पर महत्वपूर्ण (पी <0.05) अंतर के संकेत दिए। कच्चे चावल, उड़द की दाल, ज्वार और मोरिंगा फली पाउडर (एमपीपी) के लिए प्रोक्सीमेट विश्लेषण किया गया था। एमपीपी में इस्तेमाल की गई अन्य सामग्रियों की तुलना में काफी अधिक (पी <0.05) वसा (15.5 ± 0.55%) और राख (6.37 ± 0.07%) का स्तर दिखा। क.म.ग्र.इं.इ.म. विकसित करने के लिए प्रसंस्करण चरणों का इष्टमीकृत किया गया था और एसपीएसएस पर 2^5 फैक्टोरियल प्रयोग किया गया। 32 संयोजनों के लिए संवदिक गुण, बनावट प्रोफ़ाइल और स्याही प्रिंट परीक्षण इष्टमीकृत संयोजन का चयन करने के लिए किया गया । इनके अनुकूलित स्तर के आधार पर, कार्यात्मक इंस्टेंट इडली मिक्स तैयार करने के इष्टमीकृत चरणों के बाद दो नियंत्रण तैयार किए गए थे । क.म.ग्र.इं.इ.म. की प्रोक्सीमेट संरचना ने नियंत्रण की तुलना में महत्वपूर्ण रूप से ($p<0.05$) उच्च प्रोटीन, वसा, राख और नमी की मात्रा दर्शाई। क.म.ग्र.इं.इ.म. के रंग और aW ने नियंत्रणों पर महत्वपूर्ण ($p<0.05$) अंतर दिखाया। जैव-कार्यात्मक गुण अर्थात कुल कैरोटेनॉयड, फेनोलिक्स, फ्लेवोनोइड, विटामिन सी, आयरन, कैल्सियम और ऐंटी-ऑक्सीडेंट गतिविधियों ने नियंत्रण की तुलना में महत्वपूर्ण (पी <0.05) उच्च मूल्यों को दर्शाया। वर्णनात्मक संवेदी विश्लेषण ने नियंत्रण की तुलना में क.म.ग्र.इं.इ.म. से तैयार की गई इडली के रंग के साथ-साथ आंतरिक शुष्कता के संबंध में महत्वपूर्ण ($p<0.05$) अंतर दिखाया, जबकि अन्य मापदंडों ने नियंत्रणों के संबंध में गैर-महत्वपूर्ण ($p> 0.05$) अंतर दिखाया। कठोरता को छोड़कर बनावट प्रोफ़ाइल के सभी मापदंडों ने नियंत्रण और क.म.ग्र.इं.इ.म. के बीच महत्वपूर्ण ($p<0.05$) अंतर दिखा। वर्तमान अनुसंधान अध्ययन का निष्कर्ष यह है कि अनुकूलित इडली के सम्वेदिक गुणों और बनावट प्रोफाइल पर हानिकारक प्रभाव डाले बिना उत्कृष्ट जैव-कार्यात्मक गुण प्रदान किए ।



(ईश्वरी ई)

List of abbreviations

DV	Daily Value	N	Newton
J	Joule	CD	Critical Difference
KJ	Kilo joule	NS	Non-significant
FMIIDM	Functional multi-grain instant *idli* mix	S	Significant
FMID	Function multi-grain *idli*	%	Percentage
CID	Control *Idli*	N. s	Newton Second
HPMC	Hydroxypropyl methyl cellulose		
CMC	Carboxy methyl cellulose		
BOD	Biologically oxygen demand		
GDL	Glucono-delta lactone		
GMP	Good manufacturing practices		
^{O}C	Degree Celsius		
kDa	Kilo Dalton		
Mg	Milli gram		
Kg	Kilo gram		
G	Gram		
µg	Micro gram		
LAB	Lactic Acid Bacteria		
Ca	Calcium		
Fe	Iron		
Mg	Magnesium		
K	Potassium		
UV	Ultra-violet		
MUFA	Mono unsaturated fatty acid		
IS	Indian standards		
NIN	National Institute of Nutrition		
AOAC	Association of Official Agricultural Chemists		
FAO	Food and Agriculture Organization		
WHO	World Health Organization		
UNU	United Nations University		
NCDC	National Collection of Dairy Culture		
TBA	Thiobarbutric acid		
RH	Relative humidity		
FTIRs	Fourier Transform Infrared Spectroscopy		
TCC	Total Carotenoid Content		
TPC	Total phenolic content		
TFC	Total flavonoid content		
ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid		
DPPH	2,2-diphenyl-1-picrylhydrazyl		
FRAP	Ferric reducing antioxidant power		
TPTZ	2,4,6-Tri(2-pyridyl)-s-triazine		
TPA	Texture profile analyzer		

CHAPTER 1

Introduction

1. INTRODUCTION

Emerging research and investigations are aiming to satisfy customers by exploring new techniques in all fields of science and technology especially in the field of food sector. An instant or ready to use product/ technology plays a vital role in the modern world as it provides an immediate solution to almost everything. Similarly, the instant food is also gaining popularity at house-hold level for working women as well as at commercial level due to its minimum handling and the requirement of less time in its preparation. However, now, consumers demand incorporation of more healthy ingredients in instant foods for enhancing the overall functional attributes of developed product; consumption of which is expected to result in physical and mental well-being. Instant foods are available in several categories, namely ready to eat, ready to cook, instant noodles, instant mixes (*idli*, dosa, upma, soup and beverages), etc. The formulation of instant mix is suggested to be an excellent approach amongst all the available instant foods due to possibility of incorporation of a range of ingredients. Further, amongst these, formulation of instant *idli* with bio-active ingredients can result in healthier product as it already contains balanced profile of amino acids coming from a combination of cereal and legume, besides being a steam cooked fermented product which further add nutritive value to the end product.

Briefly, *idli* is a cereal (rice: *Oryza sativa*) and legume (Black gram: *Phaseolus mungo*) based fermented and gluten free food of Southern part of India. The preparation of *idli* involves very simple yet lengthy procedure. Generally, soaking of 3 parts of rice and 1-2 parts of black gram/ urad dhal is done for 4 hours followed by grinding them separately, mixing batter with salt, fermentation for 22-24 hours and finally steaming. The dehulled black gram batter plays major role in fermentation as it is a natural source of microbes as well as contributes to the fluffiness of *idli* after steaming. This is due to the presence of two important components i.e. polysaccharide (arabinogalactan) and surface-active protein (globulin). These mucilaginous principles contribute to porous and soft texture of *idli*. The globulin is involved in foam forming activity during the batter formation, while arabinogalactan are involved in stabilizing activity of foam as well as providing viscogenic activity during fermentation of batter between pH 5 to 7. The functional properties of arabinogalactan is not affected by heat but could be affected by germination because of breakdown of polysaccharide (Sucheelama and Rao, 1974). The natural fermentation of batter

produces carbon dioxide which results in increasing the volume of batter. The carbon dioxide is held by these two mucilaginous principles present in batter as well as in *idli*.

Idli is liked and consumed by all age groups with chutney, dried spicy mix (*idli* podi) and sambar (Agarwal *et al.,* 2000). Nutritionally, it can be used to address Protein Energy Malnutrition (PEM) due to the presence of good quality proteins and carbohydrates, besides the presence of higher amount of B-complex vitamins arising post-fermentation step, and lower amount of fat. The nutritional status and functional properties of *idli* can further be improved by incorporating or fortifying the base ingredients with other natural functional resources like sorghum and other millets. However, producing a fluffy and spongy *idli* in the presence of adjunct ingredients is a technological challenge as the gluten free grains like rice, urad dhal or millets are reported not to yield leavened products without fermentation. Moreover, formulation of instant *idli* mixes is even a more thought-provoking and challenging project in the presence of regular and adjunct ingredients. However, studies conducted earlier i.e. by Acs *et al.,* (1997); Kang *et al.,* (1997); Gambus *et al.,* (2001) and Sabanis and Tzia (2011) have revealed that production of leavened product from gluten-free grain flours was successful with incorporation of hydrocolloids like xanthan gum, guar gum, k-carageenan, hydroxypropylmethyl cellulose (HPMC), etc. These polysaccharides help to develop viscoelastic property of dough and improve gas holding capacity of gluten free dough (Sabanis and Tzia, 2011).

India has a rich heritage of traditional foods like fermented products of dairy/ cereal/ vegetables. The combination of these food groups in traditional fermented product is also expected to yield greater health benefits with superior functional properties like presence of probiotic, prebiotic, anti-oxidant and anti-microbial properties (Tamang *et al.,* 2016 and Bishnoi *et al.,* 2015). One of the very important functional millets and vegetables is sorghum and *Moringa oliefera,* respectively. Sorghum (*Sorghum vulgare* or *Sorghum bicolor*) is the gluten-free nutri-cereal which is the source of phenolic (flavonoids) and anti-oxidant compounds and possess anti-tumor effects on cancer cells (Huang and Ferraro, 1992; Shahidi and Naczk, 1995) and many other functional attributes. USA is the major producer of sorghum (USDA, 2021). However, sorghum is being used only in small amounts in USA and not as a staple cereal. On the other hand, in semi-arid area of Africa and India, sorghum is used as a human food directly. It is used in different forms and in an

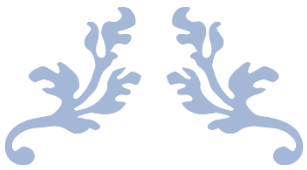
array of food products like baked bread, tortillas, porridge, steamed food, alcoholic and non-alcoholic beverages, etc. (Kulamarva *et al.*,2009).

Moringa oleifera belongs to the *Moringacea* family and the tree is known as 'miracle tree' because of its wonderful nutritional and therapeutic properties and also on account of possessing surprising properties in relation to conservation of environment as *Moringa* seeds can be used for purification of water and in turn preventing the use of chemical coagulant for the purpose (Delelegn *et al.,* 2018). Locally, it is called as drum stick tree. It is native tree of India and also exist in wild of Himalayan region of North India. Presently, it is grown worldwide in tropics and sub-tropic regions for its pods, leaves and flowers. The genus of *Moringa* includes 13 species which are distributed all around the globe. The drought tolerant species is *Moringa drouhardii* which is native of Madagascar and it can tolerate the saline soils. All parts of *Moringa oleifera* tree like flowers, pods, leaves, roots and stems possess unique health benefits and are acclaimed for their nutrient content, amino acids, anti-oxidant, anti-aging, anti-microbial and anti-inflammatory properties because of presence of high phenolic content. It is also known as a 'mother's best friend'. WHO has promoted *Moringa* since 1998 as an alternative imported food supplement for addressing malnutrition (Johnson, 2005; Singh *et al.*, 2018).

The *Moringa* leaves have been utilized in many commercial edible products like cereal products (biscuit, cake, cookies, bread), savory snacks (papad), cereal-legume based food (*idli*) and complementary foods. The pods are more popular in South Indian cuisine because of its delicious, peculiar and remarkable flavor. The pods are still being underutilized because of lack of awareness of their benefits, functional properties, appropriate method of utilization in preparation of commercial products. Therefore, an attempt has been made through this study to incorporate sorghum and *Moringa* pods in *idli* mix for addressing nutritional security with following objectives:

(1) Optimization of ingredients for development of functional multi-grain *idli* mix

(2) Evaluation of physico-chemical, bio-functional, microbiological and sensory parameters of developed product



CHAPTER 2

Review of Literature

2.0 REVIEW OF LITERATURE

In this chapter, the previous research works are collected and compiled as these will provide the status of research work already conducted on the lines of present study. Also, it will be useful in interpretation of results. This chapter will have extensive information on *idli* batter and its physico-chemical properties, health benefits of all the ingredients used for making *idli*, details of existing functional *idli* mix, sorghum and *Moringa oleifera* pods as the ingredients for formulation of instant *idli* mix and their functional qualities.

2.1 *Idli* batter and *Idli*

Idli is small, acid-leavened, white colored, steamed rice cake prepared from naturally fermented cereal-legume batter, having soft and spongy texture in nature (Krishnamoorthy *et al.*, 2013). It is a traditional product from Southern part of India and its batter is composed principally of rice (*Oryza sativa*) and dehulled black gram dal (urad dal/ *Phaseolus mungo*). Many people in India and also worldwide prefer eating *idli* because of its attractive appearance, peculiar fermented flavor, appetizing taste, spongy texture, easy digestibility, absence of anti-nutritional factors with pronounced amount of essential nutrients (Manay, 2001). *Idli* is one of the healthiest diets because of presence of balanced amino acid and energy it provides.

2.1.1 Physico-chemical characteristics of *idli* batter

The changes in physico-chemical properties of batter containing different ratios of rice to urad dal, fermented for a variable duration were studied by Balasubramanian and Viswanathan (2006). The researchers reported decrease in bulk density as fermentation time increased due to entrapment of air/ gas pockets inside the batter leading to increase in volume ranging from 1.63 to 3.1 times of its original volume. Ghosh and Chattopadhyay (2010) reported that the density ranged between 0.93 to 0.59 g/cm^3 which decreased as fermentation duration increased and volume of batter increased from 1.62 to 3.2 times of its original volume which could be due to production of lactic acid and CO_2 inside the batter. The pH ranged between 4.21 to 5.9 and total acidity ranged between 0.44 and 0.91%. This study revealed that *idli* batter showed pseudo plastic flow behavior. Batter (polished parboiled rice: decorticated black gram) rheology was measured using Brook Field viscometer having disc spindles. Shear stress value of *idli* batter was in the range of 0.22 Pa to 4 Pa (for rice: urad dal ratio of 2:1, 3:1 and 4:1, w/w) and maximum shear rate was obtained at 7 h of fermentation.

Balasubramanian and Viswanathan (2006) also revealed that the flow behavior index (which measures the deviation from Newtonian fluid or non-Newtonian fluid) of batter did not depend on fermentation time up to 6h. Consistency coefficient or index (which measures the average viscosity of non-Newtonian fluid) showed an increase as rice to black gram level increased at any fermentation time. These parameters showed that *idli* batter is a strong non-Newtonian fluid (pseudoplatic/ shear thinning). Manickavasagan *et al.,* (2013) found that the brown rice added *idli* batter showed shear thinning effect and also proved that the batter is non-Newtonian in nature with co-efficient (R^2) ranging from 0.8521 to 0.9242 for unfermented batter and ranged from 0.9657 to 0.9856 for fermented batter in Casson model.

2.1.2 Effect of ingredients on characteristics of *idli*

2.1.2.1 Rice variety for *Idli*

Rice plays a major role in the formation of good quality of *idli*. The rice variety possessing low amylopectin is required to be chosen for batter preparation because amylose content of starch in rice contributes to the sourness, tenderness, glossiness, cohesiveness and texture of rice and rice-based products (Kaw and Mabesa, 1987). It has been reported that the lactic acid production by bacterial fermentation was higher in high and intermediate amylose variety rice-based *idli* batter than low/ non-amylose variety-based batter. This is due to the presence of glucose moiety in linear form in contrast to amylopectin in which glucose is present in branched form which is difficult to break by the microbial enzymes during fermentation and hence conversion of amylopectin into acid is low. Thus, acid production in *idli* batter is also at slower rate in non or low amylose rice containing varieties.

Parboiled rice with high amylose or intermediate amylose rice variety resulted in the highest softness and was most compatible for *idli* preparation (Durgadevi and Shetty, 2014; Sowbhagya *et al.,* 1991) without stickiness among ration rice, red rice, broken rice and raw rice. It was inferred that softness of *idli* was due to the starch damage during hydrothermal processing as well as soaking of rice before wet grinding. The researchers also noticed that batter made from ration and parboiled rice showed high batter volume after fermentation and expulsion of CO_2, respectively. Thus, loss of batter volume was higher in batter prepared from ration rice. Bhattacharya (2013) noted that amylopectin rich rice or waxy rice variety gave better batter volume after fermentation though batter collapsed after steaming.

2.1.2.2 Black gram dhal or Urad dhal

Foaming is reported to be higher for black gram (5000-6000 units/g) than other legumes (Susheelamma and Rao, 1974). This is due to the presence of most crucial principle 'mucilaginous or surface-active principle' present in urad dhal which is required for leavening of *idli* batter and *idli* during fermentation and cooking, respectively. The addition of urad/ black gram dhal during *idli* preparation is important due to the lack of gluten protein in rice as presence of gluten protein is essential in producing leavened product like wheat bread and cake. However, in absence of gluten, the two important mucilaginous or surface-active principal compounds namely, arabinogalactan (a polysaccharide) and globulin (a protein) present in urad dhal play a major role in texture of *idli*. Globulin is responsible for producing foaming activity, while arabinogalactan is

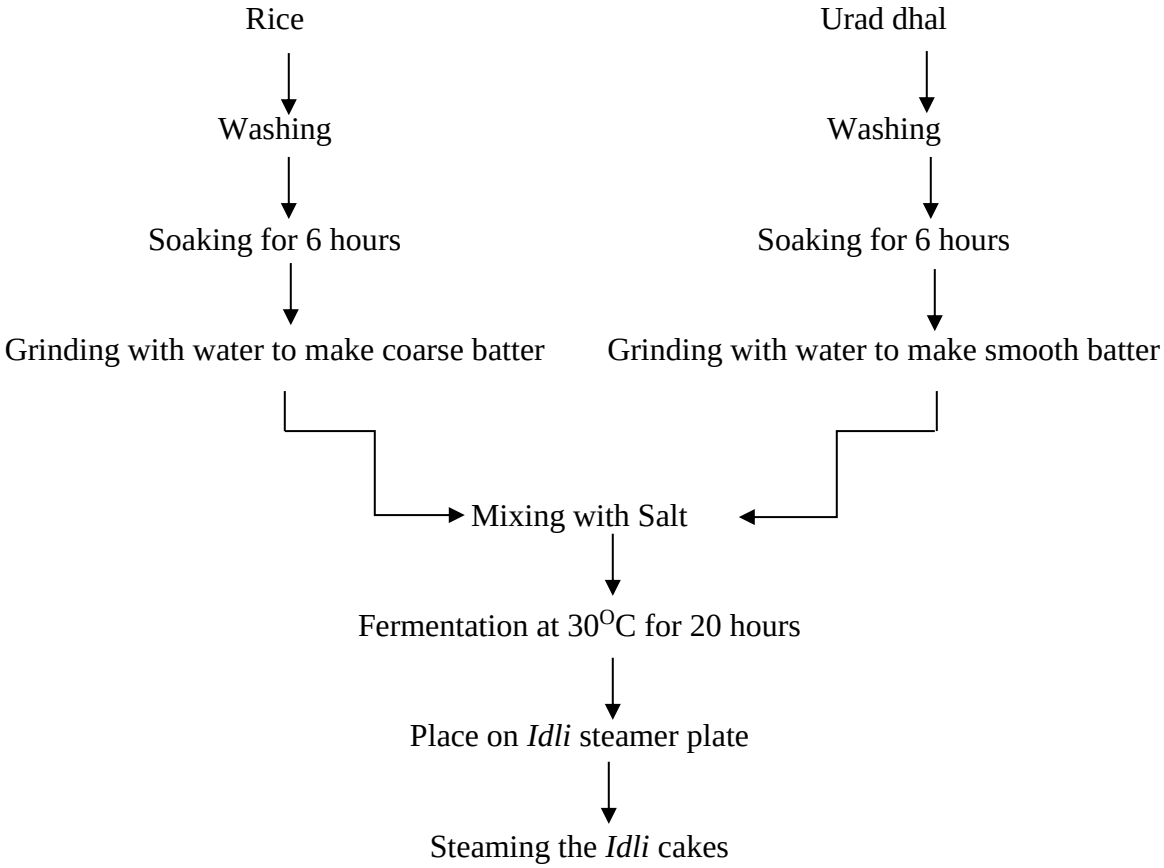


Fig 2.1. General steps involved in *idli* preparation

responsible to hold and protect the foam (CO_2) during thermal treatments; CO_2 being produced during fermentation by microbes. Yorke (2014) concluded that foam is stabilized by formation of

rigid system which is developed by crystallization, denaturation and gelatinization of arabinogalactan and globulin. This makes *idli* fluffy or spongy with maximum number of pores after steaming of batter. The leguminous substrate not only holds CO_2 but also act as substrate for microorganisms (Durgadevi and Shetty, 2012; Yorke, 2014). The most acceptable reported ratio of rice to dal is 1:1 for the preparation of *idli* (Mukherjee *et al.,* 1965). This study also revealed that increase of rice portion in *idli* batter showed a starchy flavor in the end product. *Idli* produced by using parboiled rice was better than the *idli* obtained from polished white rice. It has also been reported that black gram dal washed several times avoids growth of undesirable microorganism, which further prevents off flavor development. Sanjeev and Sandhu (1989) explored that rice-soy *idli* batters yielded *idli* rich in soluble solids, reducing sugars, total nitrogen, protein, amylases, proteinases and B-vitamins than the rice-mung dal and rice-urad dal batter because of higher content of protein and enzymes in soybean. However, among three batters, soybean added *idli* showed least acceptability. Exogenous source of α-amylase and prebiotics (xylooligosaccharides) enhanced the growth of LAB and decreased the fermentation conventional time from 14-18 h to 6 h giving soft texture to *idli*.

2.1.3 Fermentation

2.1.3.1 Natural fermentation by microbiome of *idli* batter

The primary importance of fermentation of cereals before consumption is improvement in flavour, texture, digestibility and shelf life (Durgadevi and Shetty, 2014; Nout, 2009). The legumes are naturally rich in protein than cereals, though the quality of protein is improved by fermentation (McFeeters, 1988). The complex molecules like starch and protein break down to small molecules by enzymes of microfloral amylase and protease, respectively (Kohajdová and Karovičová, 2007). Fermentation increases total acids, soluble acids, reducing sugars, microbial counts, volume, non-protein nitrogen, free amino acids, amylases and proteinases. However, it reduces many anti-nutritional factors in grains like phytate and thus detoxify the batter (Sindhu and Khetarpaul, 2001), besides improving organoleptic properties of food with peculiar tastes (Nout and Ngoddy, 1997). The fermented foods have antimicrobial effect because of the presence of lactic acid and low pH along with formation of other metabolites like hydrogen peroxide, diacetyls, propionic acid, acetic acid, CO_2, reuterin and bacteriocins that are reported to show antimicrobial activity (Shady *et al.,* 2011). Kazanas and Fields (1981) revealed that fermentation of sorghum increased amino acids like lysine or leucine, isoleucine, methionine and vitamins like niacin, thiamin and

vitamin C (Balasubramanian and Viswanathan, 2006). Agaliya and Jeevaratnam (2013) carried out molecular characterization of bacteriocinoenic LAB isolated from *idli* batter. Twenty-two different *Lactobacilli* were isolated from *idli* batter which showed Gram positive and catalase negative activity, out of which eight isolates showed maximum zone of inhibition against other LAB and various Gram positive and Gram-negative pathogenic bacteria. The isolates of batter showed good inhibition on *Bacillus cereus* and *Staphylococcus aureus*, which are reported as common contaminant of *idli* (Jama and Varadaraj, 1999). The *Lactobacilli* acted as potent inhibitor of *Listeria monocytogenes* and *Escherichia coli*. The *Lactobacilli* can tolerate and grow in salt concentration of 6.5% and acidic as well as alkaline pH but does not tolerate salt at 10%.

Fermentation temperature is one of the most important parameters affecting physico-chemical properties of batter. The temperature of 40°C support quick multiplication of bacteria along with strong reduction in pH due to increase in production of acids, increase of volume, soluble nitrogen, proteinases and vitamins B and C. This temperature favors multiplication of bacteria, acidification, leavening action and proteolytic activity. However, 28°C temperature was reported to be helpful for the multiplication of yeast and its activity, which contributed for the production of amino acids, vitamins B1 and B2 with attractive organoleptic properties (Kannan *et al.,* 2015). Reducing sugars are utilized by bacteria leading to subsequent production of acid and gas which further provide leavening.

L. mesenteroides is predominantly responsible for souring and leavening of batter at initial stage followed by growth of *Streptococcus faecalis* and *Pediococcus cerevisae*. Besides these, species like *Lactobacillus delbruekii*, *Lactococcus lactis*, *Streptococcus lactis* and numerous yeasts are also involved in fermentation. The role of *Leuconostoc mesenteroides* in leavening the batter of *idli* was studied by Mukherjee *et al.,* (1965). *L. mesenteroides* can utilize several sugars except arabinose and produce acid and dextran (mucoid-woody appearance). *Streptococcus faecalis* can digest glucose, fructose, galactose, mannose, arabinose, sucrose, maltose, lactose, raffinose and mannitol. These are common starter also for dairy products, fermented vegetables, fermented dough, alcoholic beverage and meat products and produce CO_2 enabling the batter to be in the anaerobic condition which causes batter to be leavened and acidified.

L. mesenteroides and *Streptococcus lactis* are low acid producing bacteria and are present at earlier stage of fermentation; on the other hand, high acid producing bacteria like *P. cerevisiae* appear after low acid producing bacteria yield sufficient acid and produces acid but not CO_2.

Within 24 hours of fermentation, the batter is reported to attain its maximum volume and show major changes like bacteriological and physicochemical changes. The typical aroma of fermented batter develops after 16 hours of fermentation at 30°C (Manay, 2001), but off flavor starts to develop after 40 hours of fermentation and yield batter exhibiting bland flavor, softer and thinner consistency and less glutinous-mass along with decreased sour odor at the end of fermentation. Rice semolina showed longer fermentation duration/ low volume on leavening than ground black gram batter when the two batters were kept separately under similar conditions of fermentation. The quality of *idli* was reported to improve by addition of extruded rice flour which was due to ease in fermentation because of presence of soluble carbohydrates, denatured protein and gelatinized starch which could be readily fermented by the natural microbes (Singh *et al.,* 1994).

A study conducted on the 20 different market samples revealed that fermentation resulted in predominant bacteria (10^6 - 10^8 /g, DB) and yeast (10^6/g, DB) i.e., *L. mesenteroids, S. faecalis, Lactobacillus fermentum, P. cerevisiae, Lactobacillus delbrueckii, Bacillus amyloliquefaciens* and *Saccharromyces cerevisiae, Debaryomyces hansenii, Hansenula anomala, Trichosporon beigelii, Trichosporon pullulans* and *Torulopsis candida* (Soni and Sandhu, 1989). *Lactobacilli* possesses antifungal activity, anti-tumor activity, cholesterol reducing activity, stimulate immune system, alleviate lactose intolerance and are able to stabilize the gut microbes. *Pediococcus pentosaceous* or *Enterococcus faecalis* combined with yeast *Candida versatilis* were tried as starter culture for *idli* batter fermentation (Sridevei *et al.,* 2010). Soni and Sandhu (1989) revealed that six species of bacteria (*L. mesenteroides, Streptococcus faecalis, Lactobacillus fermentum, Pediococcus cerevisae, Lactobacillus delbrueckii* and *Bacillus amyloliquefaciens*) occurred in fermented batter in the range of 10^6 -10^9 / g, while some batter sample showed presence of yeast, namely *Saccharomyces cerevisiae, Debaryomyces hansenii, Hansenula anomala. Trichosporon beigelii, Torukopsis candida* and *Trichosporon pullulans* ranging up to 10^6 /g.

2.1.3.2 Effect of fermentation on Nutritional value of *idli*

Hundred grams of *idli* is reported to yield 45 kcal of energy, 7.6 mg of protein, 0.32 mg of thiamine, 0.30 mg of riboflavin and 0.9 mg of niacin. Ghosh and Chattopadhyay (2010) reported maximum thiamine and riboflavin content of 0.73 mg/ 100g and 0.76 mg/ 100g, respectively from *idli* produced from 3:1 blend of rice: urad dal batter and folic acid was found with peak value i.e. 0.75 mg/ 100g after 10h of fermentation. The protein digestibility increased as fermentation time increased due to the production of free amino groups by hydrolyzed protein. Improved digestibility

of *idli* resulted in more acceptability for its consumption. Many researchers revealed that the methionine content increased from 10.6% to 60% during fermentation (Rao, 1961; Rajalakshmi and Vanaja, 1967; Steinkraus *et al.,* 1967 and Balasubramaniyan and Viswanathan, 2006). Further, during fermentation, vitamin B and C are increased and about 50% of phytate is hydrolyzed. The declining rate of phytate content increased as fermentation time increased i.e. 45% reduction on 1st day, 75% reduction after 2 days and 80% reduction after 3 days of fermentation (Marfo *et al.,* 1990).

2.2 Instant mix

Instant foods are also known as ready to eat or ready to cook. These are formulated using dried ingredients followed by preparation of final product with hot/ cold water or liquid ingredients with minimum amount of processing/ preparation time (Mehta and Jood, 2018) and are convenient to use. These types of dried ingredients can be stored under ambient conditions (Bishnoi *et al.,* 2015). Instant *idli* mix is convenient way of preparation of *idli* over the conventional method which is a long process.

2.2.1 Standards for ready *idli* mix

IS (Indian Standards) 2234 (1989) (Reaffirmed 2013) refers to ready *idli* mix. It states that ready *idli* mix shall mean the mix containing rice, semolina, black gram (*Phaseolus mungo*) flour, edible common salt and baker's yeast (dried) and which conforms the following requirements:

1. **Description**

The ready *idli* mix shall be in the form of a white to off-white powder, free from rancidity, insect or fungus infestation and from fermented, musty or other objectionable odour. It shall be free from added colours and flavours.

2. **Determination of freedom from dirt and extraneous matter**

According to the standards, when 100 g of water is added to 10g of mix and the concoction is stirred with glass rod for mixing of contents to form a suspension. The suspension must be allowed to stand for 2 hours and the supernatant water, surface and bottom must be examined for absence of dirt or other suspended and extraneous matter.

3. **Microscopic examination**

Microscopic examination shall not reveal the presence of foreign matter other than the ingredients as mentioned in the standards.

4. **Packaging**

The material shall be manufactured and packed in hygienic condition.

5. **Other requirements**

The ready *idli* mix is required to be packed in flexible thermoplastic films of multi-layer or monolayer, or their laminates with paper and / or aluminium foil which provide high resistance to oxygen and for effective seal. The sealing should be done hermetically with or without nitrogen.

Table 2.1 Requirements of *idli* mix

S. No	Characteristic	Requirement
1	Moisture (% by Mass max.)	12.0
2	Total ash (on DB), (% by Mass max.)	5.0
3	Acid insoluble ash (on DB), (% by Mass max.)	0.2
4	Total protein (on DB), (% by Mass max.)	12.0
5	Crude fiber (on DB), (% by Mass max.)	0.2
6	Carbohydrates, % by Mass, max	70
7	Leavening index, % *Min*	1.25

2.2.2 Ingredients and steps for development of Instant mix

2.2.2.1 Major ingredients and their pre- gelatinized forms

Native starch of rice lacks versatility which limits its applications in food industry; hence starch modification is done to improve the functional properties. Instant *idli* mix is mostly prepared from pre-gelatinized flour for decreasing preparation time and easy cooking with minimum handling. However, pre-gelatinization is affected by varieties of rice kernel, pretreatment conditions and processing methods (Chang *et al.,* 1996). It is a kind of physical modification of starch. Pre-gelatinization occurs by heating with/ without water or mechanical shear of the product like roasting, steaming, extrusion, boiling, etc followed by drying and grinding. It results in completely gelatinized starch granules leading to formation of instant starch that can be dissolved in water below the gelatinization temperature of native starch (Hong and Liu, 2018). Due to pre-gelatinization, the starch completely lacks birefringence and retains very little original granule structure.

Lai and Cheng (2004) reported that pre-gelatinized rice flour can be used as major ingredient, bulking agent or thickening agent in baby foods, instant soups and desserts. Traditionally, pre-gelatinized rice flour is produced by steaming raw rice or dried rice kernels

followed by puffing. However, this method is reported to cause contamination of pregelatinized rice due to sand puffing method and uneven heating which leads to uneven gelatinization. Another method i.e., hydrothermal treatment is also used to produce pre-gelatinized rice. In this, steamed rice is gelatinized in the presence of sufficient moisture at elevated temperature. This accompanies swelling in starch granule, loss of birefringence and crystallinity and disruption of starch granule structure (Atwell *et al.,* 1988) resulting in completely gelatinized starch rice. The starch re-orients itself because of loss of water called retrogradation (Ong and Blanshard, 1989) during drying of these hydro-thermal treated rice. Due to parboiling (hydrothermal treatment), the endosperm/ head grains quality and quantity can be increased. The authors reported that amylose content of rice also increases by hydrothermal treatment. Amylose content of starch in rice plays essential role in textural properties of *idli* (Kaw and Mabesa, 1987). Ituen and Ukpakha (2011) also reported that rice can be gelatinized without addition of water i.e. by steaming. The soaking temperature and pearling time of red and white sorghum was noted to be 73.3°C for 4.8 min and 67.9°C for 8.6 min, respectively (Gala'n and Drago, 2018), and this study revealed increase of endosperm yield of pre-gelatinized flour from parboiled sorghum.

2.2.2.2 Viscoelastic improvement by hydrocolloids and resistant starch

Lack of gas holding capacity in batter led to sedimentation in batter from instant *idli* mix and resulted in lack of soft/ spongy texture of *idli*. i.e., the obtained *idli* was hard in texture (Thakur *et al.,* 1995). Heating of starch granules cause sequence of changes like gelatinization (leaching of amylose from starch granules), pasting (swelling and disruption of starch granules) and finally retrogradation (reformation of starch granules by leaching of water). Addition of starch-hydrocolloid in to food stuffs improved texture and overall acceptability (Glicksrnan, 1986) by protecting starch granules against shear during gelatinization, increasing pasting viscosity, reducing retrogradation and syneresis (BeMiller, 2011; Wang *et al.,* 2018; Costa *et al.,* 2020). Hydrocolloids can also modify the structure of food by binding water. A crucial feature of starch rheology is viscosity. Thakur *et al.,* (1995) noted that the water holding capacity and viscosity of batter (due to air holding capacity) decides the hardness of product.

Starch also belongs to the hydrocolloid category. But, native starches have negative effects on foods like gel syneresis, retrogradation, break down, cohesiveness, undesirable gel formation and rubbery pastes (Mandala, 2012; Whistler and BeMiller, 1997). However, heating causes modifications in starch. The swelling in starch molecule by absorbing water leads to leaching of

amylose with soluble substances. The swelling of starch granules while gelatinization causes viscosity to increase and thus causes change in viscoelasticity. Starch, amylose and other polysaccharide interaction exerts force on starch granules that leads to solubilization of amylose due to break down of starch granule (Chritianson, 1982). Increasing solubility of amylose leads to improved texture of food.

2.2.2.3 Flaked rice

Resistant starch is formed during parboiling, flaking, expanding, popping, roller drying, extrusion cooking, malting and auto-claving of rice by expansion of materials (Chitra *et al.*, 2010). The resistant starch content in flaked rice is reported to be higher (Mangala *et al.*, 1999). Resistant starch is resistant to enzymatic break down due to glycosidic bond and is not absorbed by small and large intestine. Amylose rich starch is known as resistant starch and act as dietary fiber due to inability to break. Korus *et al.*, (2009) found that addition of resistant starch in gluten free bread enhanced textural properties by increasing gelatinization temperature and controlling paste viscosity. Addition of faked rice flour and expanded rice flour into black gram dhal rice batter increased cold paste viscosity of batter due to its high-water holding capacity (Ash *et al.*, 2007) which improves visco-elastic property of food.

2.2.2.4 Leavening complex system

Leavening complex system consist of two components, namely bicarbonate (a source of CO_2) and acid (which triggers CO_2 liberation with water and heat). The quantity of bicarbonates decides the amount of CO_2 released and acid controls the CO_2 liberation (Brodie and Godber, 2007). Water vapor, air, CO_2 during fermentation act as leavening agents in traditional *idli* preparation. Carbon dioxide is formed from breakdown of natural bicarbonates or sodium bicarbonate (chemical agent) with acids or natural fermentation (Penfield and Campbell, 1990). However, in instant mix, external source of leavening agents is required to be added as the mix is not kept for fermentation. Further, acid acts upon bicarbonate and produces CO_2 gas for aeration of batter. This aeration produces light weight, porous structure and enhances the texture with desirable appearance to the product. Further, gas is also held by hydrocolloid-starch complex after addition of water in *idli* mix.

2.2.2.5 Yeast extract

Yeast extract is prepared from yeast cell disruption by different techniques like autolysis and hydrolysis and soluble contents are extracted. Yeast extract is a concentrated source of

nutrients arising from yeast (Zarei *et al.,* 2016). This is used widely as food additives/ flavorings or vitamin supplements in food. Flavor enhancing capacity of yeast extract is due to presence of precursors like amino acids, nucleotides and peptides in yeast extract (Festring and Hofmann, 2010). These components are non-volatile, but on heating these provide peculiar sour flavor to foods and in turn increase acceptability of foods like soups, sauces and bakery products etc. (Nagodawithana, 1992).

2.2.2.6 Fenugreek (*Trigonella foenum-graecum*)

Fenugreek seeds are produced from hard pods and are rich in organic substance like fatty acids, protein, phosphorous, magnesium, calcium and zinc. Fenugreek act as appetizer and has trigonelline. Fenugreek has peculiar properties like anti-diabetic, anti-carcinogenic, lowering cholesterol and glucose in blood and possessing anti-microbial properties (Snehal *et al.,* 2020). It is enriched with phytoestrogens and selenium, which is beneficial for groups of population that have low levels of estrogens (Sri, 2008; Snehal *et al.,* 2020). Maurya *et al.,* (2020) found that addition of fenugreek with other pulses increased protein quality in *idli* batter and also resulted in its enhanced nutritional profile and health promoting (bowl movement) effects due to the presence of mucilaginous fiber (Snehal *et al.,* 2020). Decorticated black gram splits/ urad dhal are preferred for *idli* batter preparation. But, decortication of black gram dhal decreased the yeast content. Hence, fenugreek has been added to compensate yeast lost during decortication (Yorke, 2012). Snehal *et al.,* (2020) reported that the addition of fenugreek helps to hold CO_2 and enhances the softness of dosa.

2.2.2.7 Water

Water plays important role in cooking of *idli*. Presence of water causes swelling and gelatinization of starch. Cooking of starch granules is completed with water distributed as an ingredient and thus enhances the sensory properties of *idli* (Yorke, 2012).

2.3 Challenges in development of Instant *Idli* mix

Carbon dioxide is needed to produce fluffy and spongy *idli* from instant *idli* mix. The choice of right ingredients is needed to hold carbon dioxide in batter and *idli* from *idli* mix. Hydrocolloids and resistant starch are generally added in *idli* mix, besides simulant ingredients which are required for mimicking acidic taste like organic acids and curd.

2.4 Value addition of *idli* batter/ instant *idli* mix by fortification with other ingredients

Rapid industrialization and urbanization and changes in eating habits of people demand for ready to use snack products (Reddy *et al.,* 1982). Dried curry leaves (Murraya koenigii) incorporation was done in *idli* batter for the purpose of bio-fortification as well as for extension of shelf life (Chelliah *et al.,* 2016). The researchers used 5% level of curry leaf added to batter which increased shelf life, flavor, texture and appearance of *idli*. The functionality attributes of *idli* increased with 10 times increase in calcium than control, fiber increased by 18.6% and shelf life of batter increased from 2 to 5 days when stored at 30°C. Bulk density and viscosity of curry leaves added *idli* showed decreasing trend due to antimicrobial activity of curry leaves. Variations in textural changes occurred in fortified *idli* due to the production of acid, ionic changes in protein, and particle size variations. Firmness was the highest for treated *idli* than control; springiness was higher in curry leaf treated *idli* after 24h of fermentation. Twelve hours fermented batter showed highest acceptability. This study concluded that curry leaves could be potential raw ingredient for making novel *idli*.

Sharma *et al.,* (2018) prepared an instant *idli* mix using foxtail millet and black gram dal to save time for homemaker as well as working class of people. Millets are beneficial for humans as they are rich in vitamins, minerals, sulphur containing amino acids and phytochemicals. Thus, are termed as 'nutri-cereals'. Foxtail was used to produce nutritionally superior product in terms of protein, fiber, carbohydrate, ash and minerals. In this study, the ingredients were soaked for 24, 48 and 72 h for fermentation purpose followed by drying of ingredients at 55°C in hot air oven, coarsely grinding and storing in plastic containers. *Idli* was prepared by mixing 200ml of water in 100g of mix, and then steamed. The *idli* fermented for 48h showed smooth and soft texture, but 72 h fermentation showed adverse effect on flavor of *idli*. This study revealed that the fermentation time is an important step which determines the sensory attributes (flavor and texture) and nutritional qualities of *idli* (Nisha *et al.,* 2005).

Bishnoi *et al.,* (2015) prepared meat based *idli* mix using chicken meat powder. They prepared rice *idli* mix with 20% chicken meat powder and semolina *idli* mix with 30% chicken meat powder. The *idli* mix showed higher TBA (thiobarbutric acid) value and pH value because of oxidative break down of fat in meat on storage. These mixes showed an increase in shelf life without any undesirable microbial load up to 60 days for rice *idli* mix and 90 days for semolina *idli* mix. Nazni and Shalini (2010) developed sorghum based *idli* and evaluated its physical and

nutritional attributes. Sorghum added *idli* showed highest nutritive value with respect to calcium, iron, protein, fat and fiber; and batter volume increased in sorghum *idli* than control.

Regubalan and Ananthanarayan (2018) used mustard essential oil as bio-preservative for enhancing the shelf life of *idli* batter. The mustard essential oil was found to be effective against LAB strains and *Candida versatilis* at 80ppm and 40ppm, respectively. 0.1% of essential oil incorporation resulted in significant reduction in unwanted changes in acidity, batter volume and whey separation. All these changes resulted in reduction of sour taste, improved texture of *idli* and increased the shelf life to 5 days at 30°C and 30 days at 4°C. The authors also discussed that mustard essential oil (allyl isothiocyanate- antimicrobial component) is the only substance which showed biocidal effect at lower concentration than spices like asafetida, pepper, ginger and fennel (200-350ppm) which showed bacteriostatic effect.

2.5 Other potential ingredients for *idli* mix

2.5.1 Sorghum as a novel ingredient for preparation of *idli*

Sorghum belongs to grass family Poacea. Sorghum is regarded as 5th most important nutri-cereal after wheat, rice, maize and barley in the world (Jahan and Kamalaja, 2016) in terms of production i.e. 56.7 million tons per annum by 2017 (FAO, 2017 and Xiong *et al.,* 2019). It is grown under semi-arid area of Africa and India because of its drought tolerance ability (Ezeogu *et al.,* 2005), heat tolerant, growing capacity in infertile soil, high saline-alkaline soil and high altitudes (Rooney and Waniska, 2000). Sorghum is one of the nutri-cereals possessing highest genetic variety because more than 30,000 selections are conserved in the World Collection bank located in India (Saldivar, 2016) and also because sorghum has been utilized in staple or traditional foods of Africa and India. Sorghum is also known as jowar in India, great millet and guinea corn in West Africa, Kafir corn in South Africa, Mtama in Eastern Africa, dura in Sudan and kaoliang in China (Kulamarva *et al.,* 2009). It is classified into high and low tannin types. Brown sorghum is highly resistant to insects and birds because of presence of high tannins but shows low nutrient availabilities. On the other hand, white sorghum has low tannin content with high nutrients and it is utilized in many snacks, bakery application like bread, cookies, etc., and in beverages like beer.

2.5.2 Nutritional and chemical constituents of sorghum

Sorghum contributes majorly to the diets of millions of people in Africa and India with its high protein (gluten free) and energy yielding ability. Albumin and globulin are the proteins present in sorghum at the level of 10-30% but not been characterized well (Bean *et al.,* 2019).

Henley *et al.,* (2010) mentioned that like other cereals storage protein of sorghum i.e. kafirins is also low in lysine content due to small sized germ. Pezzali *et al.,* (2020) noted that protein and starch were significantly ($p<0.05$) high and low in white sorghum and red sorghum flour, respectively. Tasie and Gebreyes (2020) determined moisture (9.66 to 12.94%), ash (1.12 to 2.29%), fat (2.48 to 4.60%), fiber (2.17 to 8.59%), carbohydrate (67.56 to 76.42%), protein (8.20 to 16.48%), calcium (67.159 mg/100g) and iron content (14.018mg/100g) in different varieties of sorghum.

Table 2.2 Nutritive value of sorghum per 100g by NIN (Langvah *et al.*, 2017)

Chemical constituents	Concentration (g)	Chemical constituents	Concentration (g)
Moisture	9.01±0.77	Total fat	1.73±0.31
Carbohydrate	67.68±1.03	Total fiber	10.22±0.49
Protein	9.97±0.43	Soluble fiber	1.73±0.40
Ash	1.39±0.34	Insoluble fiber	8.49±0.40

2.5.3 Digestibility of Sorghum proteins

Functionality of sorghum protein is not exposed as functional regions are entrapped in protein bodies. The digestibility and solubility of protein (kafirins) is reduced by wet cooking due to the formation of oligomeric/ polymeric proteins and disulfide bonds (Bean *et al.,* 2019) or presence of polyphenols, lipids and cell wall components. Kafirin protein also affects the gelatinization of starch and decreases the starch digestibility (Stonestreet *et al.,* 2010; Duodu *et al.,* 2002). But, dry cooking like roasting do not alter the protein of sorghum. Thus, it does not affect the digestibility. Though white sorghum lacks condensed tannins, it does not affect protein digestibility. The techniques like soaking, fermentation and cooking reduces phytic acid present in sorghum leading to increase in protein digestibility. Elkhalifa *et al.,* (2004) noted that fermentation of sorghum grains up to 28 hours increased in-vitro protein digestibility, iodine absorption and decreased resistant starch content due to changes in protein and also exposed starch to digestive enzymes during digestion.

2.6 *Moringa oleifera*

Moringa oleifera belongs to Moringacea family (Anwar *et al.,* 2007) and its pods (vegetable) fall under Brassica order. The various names of *Moringa* tree are drumstick tree, horse

radish tree and West Indian Ben. Totally, 14 species (National Research Council, 2006) of *Moringa* are grown in the world, out of which most are grown in Asia and Africa (Padayachee and Baijnath, 2012) especially sub-Himalayan areas of India, Pakistan, Afghanistan and Bangladesh (Fahey, 2005; Kasolo *et al.,* 2010). Later, it infiltrated/ entrained in to warm countries i.e., tropical area and sub-tropical (Fuglie, 2001; Kasolo *et al.,* 2010; Udikala *et al.,* 2017; Mallenakuppe *et al.,* 2019; Sandeep *et al.,* 2019). Among 14 species, *Moringa oleifera* is grown and utilized widely (Bichi, 2013). India is the largest producer of *Moringa* worldwide with maximum production from Andhra Pradesh, Karnataka and Tamil Nadu states in India (Bichi, 2013; Sandeep *et al.,* 2018).

2.6.1 Nutritional properties of different parts of *Moringa oleifera*

The research on different parts confirmed that leaves, pods, seeds, flowers, stems, roots and gums of *Moringa* tree possess applications in several fields like medicinal/ pharmaceutical, therapeutic, nutraceutical, food for humans as well as feed of animals, bio-fuel, cosmetics and environmental conservation (Fuglie, 2001; Padayachee and Baijnath, 2012; Fuglie, 2001). Pods and leaves are known for their nutrient dense quality with respect to minerals like iron and zinc, beta- carotene, vitamin C and all essential amino acids (Busani *et al.,* 2011). Leaves of *Moringa oleifera* are used mostly as food and medicine as it is concentrated source of bio-active components such as total and soluble protein content of 29.1g/100g and 25.9g/100g, respectively; minerals like calcium (16046.7 µg/100g), iron (97.9 µg/100g), potassium (17450 µg/100g), magnesium (2833.8 µg/100g), zinc (29.1 µg/100g) (Olson *et al.,* 2016); vitamin C (2.18mg.AAE/g) (Shanmugavel *et al.,* 2018). Fejer *et al.,* (2019) found vitamin E (178.10mg/kg) and essential fatty acids (oleic, palmitic and linolenic acids to be 25.01%, 24.84% and 24.71%, respectively) contents in *Moringa* leaves. Shanmugavel *et al.,* (2018) determined total polyphenols (627mgGAE/100g) and total flavonoids (22.16mgQE/100g) which included myrecytin, quercetin, kaempferol in *Moringa* leaves. Kasolo *et al.,* (2010) and Sankhalkar and Verneker (2021) revealed the presence of tannin, saponin, flavonoids, terpenoids, alkaloids and anthraquinones in leaves. Leaves are utilized to cure malnutrition in children. Kasolo *et al.,* (2010) carried out a case study and reported 24 medicinal uses of *Moringa oleifera* leaves among Ugandan rural communities i.e., in reducing HIV/ AIDS related symptoms i.e., syphilis, bronchitis, external sores, malaria, hypertension, diabetes, colitis, gastritis, etc., The anti-microbial activity, anti-carcinogenic, anti-mutagenic and anti-inflammatory properties are also reported based on *in-vitro* studies. Leaf powder has been used for washing

hands (Torondel *et al.,* 2014) because of its anti-bacterial properties (Rahman *et al.,* 2009; Okorondu *et al.,* 2013 and Gomashe *et al.,* 2014).

Flower of *Moringa* occurs in yellow color streaked with pink or red, which are commonly eaten as fruits for medicinal purposes (Singh *et al.,* 2000). As per Sankhalkar and Verneker (2021), the total phenolic content and total flavonoids of flowers were 1.08mgGAE/ml and 4.41mgQE/ml, respectively. The extract of flower was screened for phytochemicals and showed phenolic compounds, alkaloids, tannins, steroids, proteins, amino acids, vitamin C, carboxylic acid and amide in ethanol as well as hexane extract; flavonoids in hexane extract; terpenoids and glycosides in ethanol extract (Suryawanshi and Umate, 2018). Divya *et al.,* (2019) studied flower of *Moringa oleifera* and noted that total ash, water soluble ash, acid insoluble ash, water soluble extractives and alcohol soluble extractives were 0.069%, 0.053%, 0.0035%, 33.35% and 17.6%, respectively. *Moringa oleifera* is not only used as food but also used in therapeutic, medicinal, cosmetics, bio-fertilizers and pollution control (water purification) applications. Every part of *Moringa oleifera* is used for wider applications.

The fruits/ pods have three-sided capsule and inside pods seeds are present which are covered by three whitish papery wings. Entire pods are cooked/ boiled with sambar recipe, salads and curries for special meals. Infants are fed with boiled pulp of pods. Pods are generally believed to be capable of acting as parasitic drug (anthelmintic), used in treating joint pains, and liver and spleen infections. It is mainly used by lactating mother to increase the quantity of breast milk (Ponnuswami, 2012; Masih *et al.,* 2019).

Moringa pods contain edible parts of pulp and seed, the woody outer portion is not edible as it is rich in fiber and hard in nature. Joshi and Jain (2011) analyzed proximate composition of *Moringa* pod powder and reported it to be 10.18g protein, 5.43g fat, 5.09g ash and 4.85g fibre. *Moringa* pods contain high amount of iron, calcium, phosphorous, vitamin C and fiber (40% soluble dietary fiber) (Patel *et al.,* 2017). Edible portion of pods are rich in calcium (30mg/ 100g), phosphorous (110mg/ 100g), iron (5.3mg/ 100g) and vitamin C (120mg/ 100g). Fresh pods and seeds contain a good amount of oleic acid.

Seeds are white in colour inside the pods and surrounded with papery material. They are generally removed from highly matured pods, can be fried/ boiled and eaten like peas. Seeds are rich in protein (34% to 37.48%) and low in fat and carbohydrates and are significant sources of

minerals like calcium (357.78mg per 100 g), phosphorous (127.6mg per 100g) and iron; and vitamins like A, B and C, (Price, 2007; Olushola, 2006; Bolarinwa *et al.,* 2017; Garza *et al.,* 2017; Manju *et al.,* 2018; Harimbi and Muyassaroh, 2017; Dwi *et al.,* 2018). Mgbemena and Obodo (2016) also revealed that seeds of *Moringa* contained 17.94% protein, 12.60% fat, 2.61% calcium and 0.95% potassium. Seeds possess sulphur containing amino acids like methionine and cysteine at higher amount, so can be used along with legumes (which are low in methionine). Gazara *et al.,* (2017) have studied seeds protein isolates and total hydrolysates produced by proteolytic enzymes such as pepsin, trypsin and chymo-trypsin for determining its bio-functional properties. They reported that amino acids of raw seed flour like glutamic acid, aspartic acid, arginine, isoleucine, phenylalanine and leucine were present at 17.87g, 15.70g, 8.28g, 3.83g, 4.23g and 3.27g level per 100g, respectively. Seed contained high magnesium content over the magnesium content of leaves (Gopalakrishnan *et al.,* 2016; Bolarinwa *et al.,* 2017). A lot amount of anti-nutritional contents are also reported in seeds.

Seeds contain excellent amount of protein and fat, majorly mono unsaturated fatty acids (MUFA) i.e. predominantly oleic acid present at ~70% level (Abdulkarim *et al.,* 2005; Ferreira *et al.,* 2008), saturated fatty acids (palmitic, stearic, arachidic and behenic acids) and reasonable amount of carbohydrates and minerals. The oils from seeds can be extracted at home by simple extraction process. The seed oil is an excellent source of oleic acid (73%). *Moringa* seed oil is highly stable as it contains oleic acid and behenic acid (Ferreira *et al.,* 2008; Manju *et al.,* 2018). The seed meal of *Moringa* contain organic matter (95.55%), crude protein (43.26%), crude fiber (12.08%), and ash (4.45%). González *et al.,* (2017) found that the protein isolates of *Moringa* seed contained protein (49.67%), ash (2.37%), fat (32.65%), fiber (7.78%) and moisture (1.9%) with pH 11 and pI 4. The bioactive components in seeds revealed osmotic balance effect, balancing hormonal changes, regulated enzymatic activity and various metabolic activities (Udachukwu *et al.,* 2018).

Seed can be used as an alternative source of leguminous plant because of high quality protein, oil and anti-oxidants (myricetin) (Fahey, 2005). Seeds showed anti-microbial activity, anti-tumor, anti-inflammatory, anti-pasmodic, diuretic and mosquito larvicidal activity (Eilert *et al.,* 1981; Madsen *et al.,* 1987; Ghebremicheal *et al.,* 2005; Bharali *et al.,* 2003; Cáceres *et al.,* 1992 and Ferreira *et al.,* 2008). The roasted seeds are included in diet like peanuts. Oil extracted

seed cake is enriched with protein. González *et al.,* (2017) reported proximate composition of defatted seed flour as 37.48% protein, 3.73% ash, 26.54% fat, 5.65% fiber and 5.32% moisture. The oil is highly thermo-stable than peanut oil for cooking due to presence of anti-oxidant and saturated fatty acids (Anwar *et al.,* 2003; Abdulkharim *et al.,* 2005; Saa *et al.,* 2019). Foaming property and water absorption capacity of seed flour was reported to increase as fermentation time increased (Ijarotimi *et al.,* 2013). The food applications requiring high foam stability can use defatted *Moringa* seed flour at high pH (9) and high concentration (4% W/V) (Mune *et al.,* 2016). Seeds not only purify water, but also reduce the total coliforms count (Amagloh and Benang, 2009).

2.6.2 Applications of *Moringa oleifera* in formulation of novel foods

Moringa oleifera is commonly known as 'drumstick tree' or 'horsedish tree' or 'miracle tree'. Moringa tree's different parts can cure more than 300 diseases (Gowrishankar *et al.,* 2010; Ganguly, 2013). Leaves, pods, flowers and roots of *M. oleifera* are used for edible purposes and pods are much popular for its distinct flavor and utilized in south Indian cuisine and leaves are used to combat the malnutrition (Anwar *et al.,* 2007; Nadeem *et al.,* 2020). *Moringa* leaf powder is a concentrate source of nutrients; *Moringa* seed oil is used as cosmetic and cooking oil for salad dressing, cake from oil extracted seed is used for bio fertilizer production and used in purification of water. Tea from leaves and leaves in cookies/ noodles/ biscuits/ bread are used as fortificant for iron and calcium salts (Moyo *et al.,* 2011; Abd Rani *et al.,* 2018).

Seed oil of *Moringa* has fabulous quality because of presence of anti-oxidants, namely flavones and myricetin (Fahey, 2005; Patel *et al.,* 2017). According to NIN (2017), *Moringa* pod contains potassium (417mg), calcium (33.3mg), magnesium (38.1mg), phosphorous (52.87mg) and iron (0.73mg). The entire pods are cooked and used in sambar/ salad/ curries as special meals. Infants are fed with boiled pulp of pods. It is mainly used by lactating women to increase breast milk production (Ponnuswami, 2012; Masih *et al.,* 2019). Oliveira *et al.,* (1999) compared its essential amino acid profile with the FAO/WHO/UNU scoring pattern requirements which showed lack of lysine, threonine and valine. But the content of methionine + cysteine was exceptionally higher and closer to human milk, chicken egg and cow's milk. The seed extract fed along with diet to rat caused loss of appetite, impaired growth, swelling of internal organs and atrophy of thymus and spleen. However, this study was carried out using raw pulverized seeds and thus the observations could be a result of anti-nutritional factors in pods. However, these could be reduced

after suitable processing treatments before using pods in food applications. *Moringa* pod powder was utilized in lassi for its value addition (Mistry *et al.,* 2018). The optimized product contained 1.63% pod powder. Two hundred fifty grams of lassi was evaluated to provide nutrients like calcium (25% DV), iron (12%DV), potassium (10%DV) and protein (10% DV). This lassi also contained a considerable amount of vitamin C and fiber. Extract of seed showed inhibition effect against *Bacillus sphaericus*, *Mycobacterium smegmatis*, *Staphylococcus aureus* and *Alcaligenes faecalis* (Rockwood *et al.,* 2013).

Dehydrated drumstick powder extensively is used in many curries and food preparations, since it gives unique palatable taste and is rich source of glutamic acid (Ramachandran *et al.,* 1980; Patel *et al.,* 2017). Shunmugapriya *et al.,* (2020) identified the flavor compounds in *Moringa oleifera* pod powder and soup mixes from *Moringa* pods. They denoted that the acceptance or rejection level of *Moringa* pod powder and related value-added product depend on their flavor. Flavor, a combination of odour, taste and mouth feel, is developed by aromatic substances which develop during metabolism and are subsequently modified by processing. During processing, reactions leading to produce flavor are pyrolysis of amino acids and peptides, carbohydrate degradation, interaction of sugars with amino acids and peptides, breakdown of ribonucleotides and lipids (Shahidi and Naczk, 1995). This study found the presence of volatile compounds in pods. The authors used two different powders for soup mix i.e. first powder was prepared from inedible portion (woody), pulp and seed i.e. whole pod and secondly, powder was prepared only with pulp portion (edible). Powder also contained different agents like corn flour, thickening agents and flavouring agents (skimmed milk powder, soy flour, pepper powder, cumin seed powder and onion powder). GC-MS was used for analysis of flavoring compounds. They determined that major compounds in whole pod powder were 2-pentanethiol, 2-methyl and 3-hexen 2-one. Pulp powder was rich in 3-hexene2-one, 5-hydroxymethylfurfural, benzeneacetonitrile, 4- hydroxyl and 2- dimethyl trimethylsilylmethyl. Fresh soup mix from whole pod powder was high in 2-pentanethiol, 2-methyl, 1-pentanol, 2, 2-dimethyl, 3-hexe2-one and piperine. Fresh soup mix from pulp powder majorly contained 2-pentanethiol, 2-methyl, pentane, 3- ethyl-3-methyl, 3-hexen2-one and piperine. The flavor analyses was conducted after 180 days of storage in metalized polypropylene material. Pod powder soup mix contained 2-pentanethiol, 2-methyl, 3- hexen2-one, n- hexadecanoic and oleic acid. In case of soup mix from pulp powder, mostly butanoic acid, 2- ethyl-2-methyl, pentane, 3- ethyl-3-methyl, 3-hexen2-one, nhexadecanoic

acid and oleic acid was present. Some compounds in mix denoted for its anti-microbial, antioxidant, anti-inflammatory properties etc., For example, octadecadienoic acid expressed antiinflammatory, hypochloesterolemic and anti-arthitic activity (Rani *et al.*, 2009; Uma *et al.*, 2009; Ponnamma *et al.*, 2012). This study finally concluded that the compounds like octadecadienoic acid, n-hexadecanoic acid, squalene and piperine possess antioxidant, antimicrobial, anticancer, antidiabetic and anti-inflammatory properties.

Bolarinwa *et al.,* (2017) analyzed nutritive value and acceptability of bread fortified with *Moringa* seed powder (dehulled). Different levels i.e., 0-20% of seed powder was used for supplementation. Moisture content was reduced after addition of seed powder leading to longer shelf life. Protein content of bread was increased as such on addition of *Moringa* seed powder, 20% of seed powder fortified bread had the highest protein content (13.5%), while the un-fortified bread had the lowest protein content (8.6%). This was due to protein present in *Moringa* seed. But this protein content (8.6% - 13.5% increment) was low compared to *Moringa* leaf fortified (17-88% increment) bread (Oyeyinka and Oyeyinka, 2018) and potato fortified with soy flour bread, which was due to level of the fortificant addition. Relatively mineral/ ash content of bread had increased (56% to 64% increment) with increase in seed powder concentration due to higher ash (4.1%) content of *Moringa* seed (Abiodun *et al.,* 2012) except zinc (because seed contain low level of zinc). There was no significant difference in sensorial attributes between the control (100% wheat flour) / unfortified bread and 5% fortified bread. *Moringa* seed fortified bread had higher palatability level because of high fat content (7.3% - 15.8%) of seed than un-fortified bread. Crude fiber content was also found to have increased, while carbohydrate content decreased (from 60.5% to 48.4%) as the seed level increased. Vitamin A content of approximately 4.3-8.5 mg/ 100 g was present in the fortified bread, which was higher than un-fortified bread. Five percent level contributed to a most acceptable category compared to all levels of seed fortified bread. This study concluded that *Moringa* fortified bread could be consumed as supplement in developing countries.

2.6.3 *Moringa* seed fortification in raw milk

Now-a-days, the usage of cheap, eco-friendly and biofriendly plants in food industries is increasing due to their functional properties like antioxidant and antimicrobial, which enhances the shelf life and safety of raw milk. Ekpo *et al.,* (2019) evaluated the impact of dehulled *Moringa oleifera* seed extract (MSE) on bacterial load and sensory evaluation of raw milk. The study used 0% (control), 10%, 20%, 30% and 40% of MSE in raw milk. Microbial analysis was carried after

24hr, 48hr and 72hr of incubation at room temperature. The study revealed that MSE plays function through pasteurization thereby serving as an available source of microbe-free of raw milk. Average total viable count and total coliform count were higher in control and was low in 40% MSE added milk at 0 hour. Increasing the level of MSE led to decrease in the count of viable bacteria and total coliforms in raw milk, which proved the anti-biotic effect of *Moringa* seed. These counts increased further with increasing time of incubation and the highest count was in control than MSE added milk especially at 72nd hour of incubation. Microbes like *Bacillus*, *Shigella*, *E. coli*, *Serratia* and *Aeromonas* were present in MSE treated raw milk. The sensory evaluation of this study showed highest overall acceptability for control sample than MSE treated milk. However, based on colour and flavor parameters, 10% MSE added milk received the highest score, and lowest score was attained by 30% and 40% MSE added milk as high concentration adversely affected the taste of raw milk. However, there was no adverse effect on colour, taste and overall acceptability of raw milk by MSE. This study concluded that addition of 40% MSE could reduce the microbial load of raw milk from 0 to 24 hours.

Masika and Afoloyan (2002) reported that gram negative bacteria are most resistant to water extract of *Moringa* leaf. Paz *et al.,* (1995) and Martini *et al.,* (1998) reported that the plant-based water extract does not show much activity against bacteria than other solvents as active compounds in seeds do not solubilize in water completely.

2.6.4 Application of *Moringa* seed in yoghurt

Probiotic yoghurt is a dairy based beverage. Apart from its nutritive values, consumer also expect good physico-chemical quality with least syneresis (Domagala *et al.,* 2013). The texture of yoghurt is improved by increasing TSS content / using thickeners (Zhang *et al.,* 2019). *Moringa* seeds have coagulant activity because of presence of soluble proteins (Baptista *et al.,* 2015) which are natural cationic polyelectrolyte. Cardiness *et al.,* (2018) studied yoghurt thickness after addition of *Moringa oleifera* seed saline extract obtained through ultrafiltration. Different fractions of seed additive were used i.e., *Moringa* integral additive (MIA- without ultrafiltration), *Moringa* concentrate additive (MCA) and *Moringa* permeate additive (MPA), and last two were obtained from ultrafiltration. Among these, MIA had high amount of protein and total phenolic content (12.78 mg EAG/ ml). MPA had the lowest protein because protein has high molecular weight, thus is difficult to pass through filter under low pressure (30kDa) and MCA had higher total phenol which was bound to protein (Han *et al.,* 2011).

Protein and phenolics form the bond like hydrogen, hydrophobic, ionic and covalent bonds. Phenolics in milk cause the interaction between phenolics with hydrophobic surface of milk protein, which can reduce the hydrophobic interaction between the amino acid side chains. This reduction of hydrophobic groups could affect in a decreasing of water binding (Silva *et al.*, 2017). Therefore, the addition of *Moringa* seed fraction interfere with the technological characteristics of the yoghurt. The titratable acidity ranged from 0.8 to 1.11 g lactic acid/ 100g under storage period which was in the range as per Codex Alimentarius Commission (2010) i.e., 0.6% -1.5%. MIA and MPA showed higher ranges of titratable acidity. The pH of yoghurts was higher and less bacterial activity was observed as compared to control, because of presence of *Moringa* additive/ non-fat solids was observed (Kiros *et al.,* 2016). But, during storage period the pH got reduced and the significant difference occurred at 3rd week of storage with control. Protein content was higher using MCA and lowest at MPA but control had lowest protein amongst all. Syneresis was higher in control, but lowest in MPA yoghurt. MPA obtained by 1.5 bar pressure in concentration of 1.5% (v/v) in yoghurt effectively reduced the migration of whey in surface and improved the texture of yoghurt. Further, high phenolics cause large pore size in gel matrix by inducing structural changes in gels, which led to higher syneresis (Silva *et al.,* 2017) but MPA showed lowest total phenolics among *Moringa* additive, so caused lowest level of syneresis. More compact network was formed than control which exhibited open spaces and revealed less cohesion between protein and formation of casein network, but MPA added yoghurt showed the cohesive network without empty area. SEM micrograph also revealed the presence of more cohesive structure on MPA added yoghurt. Thus, low level of syneresis occurred and showed greater stability under storage period. Finally, this study revealed that *Moringa* seed additive can act as thickening agent and improved the technological characteristics of food systems.

Moringa seed oil/ chitosan nanoparticles embedded gelatin nanofibers were used as antimicrobial packaging for cheese. Nanofibers were successfully prepared and used and the oil released from nanoparticles declined because of encapsulation and packaging material possessed high antimicrobial activity against *Listeria monocytogenes* and *Staphylococcus aureus*. So, this packaging could be feasible in food preservation (Lin *et al.*, 2019).

2.6.5 Pharmaceutical properties of *Moringa* pods

Mehta and Agrawal (2003) revealed the effects of *Moringa oleifera* on the lipid profile of normal and hyper-cholesterolaemic rabbits. The rabbits were fed *Moringa* fruit and lovastatin (a

drug used to lower LDL cholesterol) along with bananas. The treated rabbits showed reductions in LDL cholesterol, liver lipid profile, and lipid was excreted in feces**.** Hence, *Moringa oleifera* fruits proved the hypolipidemic effects. Clinical trial also showed similar results (Patel, Personal communication, 2001). Guzman *et al.,* (2009) reported that an extract of the pod of *Moringa oleifera* can inhibit LPS (lipopolysaccharide) induced tumor necrosis factor, IL-1β and IL-6 production in murine macrophage cell line.

Sharma and Paliwal (2014) evaluated the effect of *Moringa oleifera* pods and isolated saponin from pods on chemoprevention. Renal carcinogenesis was induced by 7,12-dimethylben[a]anthracene (DMBA). Hydroethanolic extract of *Moringa* and isolated saponin attenuated the induced renal carcinogenesis in mice. DMBA administration had increased the level of xenobiotic enzymes with metabolizing the polycyclic aromatic hydrocarbons (carcinogens) especially renal malondialdehyde and reduced the renal antioxidant enzymes i.e., glutathione oxidase along with decrease in the renal glutathione. The uptake of *Moringa* extract hindered the renal oxidative stress and toxicity. Phytochemicals in *Moringa* leaf can quench the ROS and can regenerate the membrane bound antioxidants.

Bharali *et al.,* (2003) revealed the chemomodulatory effect of *Moringa* pods/ drumsticks on hepatic carcinogen metabolizing enzymes, antioxidant parameters and skin papillomagenesis in mice. Hydro-alcoholic extract used in papillogenesis was induced in mice skin using 7,12 dimethylben[a]anthracene. The incubation period results revealed that cytochrome b5, cytochrome P450, catalase, glutathione oxidase, glutathione reductase, acid soluble sulfhydryl content increased and significant reduction of malondialdehyde level occurred when extracts of *Moringa* pods was used. The percentage levels of papilloma's decreased in mice. These changes expressed that chemo-preventive potential of Moringa extracts against carcinogen.

Guon and Chung (2017) identified the induction of apoptosis by fruits/ immature pods of *Moringa oleifera* in human melanoma cells. The treated melanoma cells showed increased activities of cleaved caspase-9 and caspase-3 (both executing apoptosis). This caused the enhancement of MAPK (mitogen activated protein kinase) phosphorylation- a pathway showing apoptosis. Thus, fruits of *Moringa* possess the pro-apoptotic activity.

2.6.6 Pharmaceutical properties of *Moringa* seeds

Abdulrahman and Haddad (2015) revealed the diabetic effects of low doses (50 and 100mg/ kg of body weight in diet) of seeds of *Moringa oleifera* by streptozaotocin induced diabetes and

diabetic nephropathy in male rats. The lipid peroxide in serum and antioxidant enzymes (catalase, superoxide dismutase and glutathione) was reduced in *Moringa* fed rats, but increased in non-treated diabetes induced rat. IgG, IgA, Interleukin-6, fasting blood sugar and glycosylated hemoglobin were increased in diabetic rat (non-treated), but decreased during 4 weeks in *Moringa* fed rats. Higher dose of *Moringa* seed powder was more effective in ameliorated kidney functions in rats. Sodium and potassium levels were better by feeding the lower doses of seed powder. Body weight increased by providing seed powder to the diabetic rats during the 4 weeks of study.

Water consumption, food intake and food efficiency ratio decreased in *Moringa* seed powder fed rats, but increased in non-treated diabetic rats. Treated rats had normal kidney tissues than non-treated diabetic rats. Anti-microbial activity was determined by water soluble lectin from *Moringa oleifera* seeds against the corrosive and pathogenic bacteria. Bio-corrosion is corrosion occurring by metabolites released by microorganisms especially bacteria in biofilms on metal surface in presence of oxygen (Moura *et al.,* 2015). This issue causes major problems in oil and petroleum industries due to formation of biofilms in pipeline and filters. Some corrosive bacteria can act as pathogenic bacteria like *Bacillus cereus, Serratia marcescens*, *Pseudomonas fluorescens*. Lectin possess bacteriostatic and bacteriocidal effects (Sa *et al.,* 2009; Costa *et al.,* 2010; Gomes *et al.,* 2013; Ramos *et al.,* 2014) and show insecticidal properties. The anti-bacterial effect of lectin is due to the interaction with carbohydrates and glycoconjugates in the cell wall of bacteria (Paiva *et al.,* 2010). Seed lectin extract has higher hemagglutinating activity. Lectin showed bactericidal effect *against B. pumillus, B. megaterium, Ps. fluorescens and Ser. marcescens*. The growth was not observed for *Bacillus cereus* and *Bacillus sp*, because of clustering of cells and the same effects were identified in minimum inhibitory concentration (MIC) assay using lectin extract. Main role of lectin on anti-microbial activity is formation of pore on cell wall leading to the leakage of cellular content. This lectins effect was highly efficient against *Ser. marcescens* bacteria and biofilm degradation effect was also expressed (Klein *et al.,* 2015).

Moringa oleifera leaves, seeds and bark extract having anticancer effect analyzed against breast and colorectal cancer cell lines (Al-Asmari *et al.,* 2015). Cell lines treated with *Moringa* leaves and bark extracts showed significant reduction in cancer cells. *Moringa* leaves and bark extract treated cancer cell reduced significantly than seed extract and apoptotic cells count increase was found in apoptosis assay of treated breast and colorectal cancer cell lines. But, by using seed extract no apoptotic cell were identified in treated cell lines. The extracts were analyzed in GC-

MS and revealed the anticancer compounds like eugenol, isopropyl isothionate, dallose and hexadeconoic acid ethyl ester. Hence, this study expressed the anti-malignant properties of leaves as well as bark.

Giacoppoa *et al.,* (2017) isolated isothiocyanate from *Moringa oleifera* seeds and showed potential anti-inflammatory effect in the urine of treated mice for sub-acute Parkinson's diseases. Isothiocyanate (4- α -L-rhamnopyranosyloxy) benzyl glucosinolate was formed by bioactivation using myrosinase from glucomoringin (4-α-L-rhamnopyranosyloxy) benzyl glucosinate. Subacute Parkinson's disease affected mice was treated with *Moringa* with glucomoringin for a week. Inflammatory pathway modulation, oxidative stress and apoptotic pathway were suppressed by *Moringa* with higher efficacy than glucomoringin in in-vitro assay. This study revealed that *Moringa* could possibly produce the bioactive compounds by hydrolyzing glucomoringin in seed of *Moringa* by myrosinase.

Minaiyan *et al.,* (2013) revealed anti-inflammatory, immune-modulatory and anti-oxidant effects of *Moringa oleifera* seeds hydro-alcoholic extract (MSHE) and its chloroform fraction (MCF) on 4% acetic acid induced acute colitis in rats. Study used three doses i.e., 50, 100 and 200mg/ kg of body weight and fed orally to male Wistar rats 2h before ulcer induction and continued for 5 days. Prednisolone was used as reference. Bio-phenols possess the anti-inflammatory (Mahajan *et al.,* 2007) and anti-oxidant (Shaila *et al.,* 2010) properties in *Moringa* abundantly and are beneficial for inflammatory bowel diseases. *Moringa* suppresses the free radicals (Shaheen and Annette, 2011), prostaglandin biosynthesis (Mehta and Agarwal, 2008), cytokines (Mahajan *et al.,* 2007) and leukotriene biosynthesis. These studies concluded that the *Moringa* seed extract possesses anti-inflammatory properties similar to glucocorticoids.

The present study was envisaged considering the review of literature collected on various aspects. The focus of the present study was to develop a functional instant *idli* mix enriched with milk protein, a nutri-cereal, i.e. sorghum and a vegetable i.e. *Moringa*. These ingredients were chosen so as to improve the nutritional quality of *idli*s in terms protein, ash and bio-functional ingredients over the conventional *idli* mix available in market and prepared solely from rice and urad dal.

CHAPTER 3

Materials and Methods

This chapter explains the materials and methodologies which are utilized for technological, analytical and statistical aspects of functional Multi-grain Instant *Idli* mix and *Idli* development. The details of equipment used and methods adopted in the study are mentioned in this chapter under suitable titles and subtitles.

3.1. Materials

3.1.1 Chemicals

H_2O_2 (Merck Specialities Private Limited, Worli-Mumbai, India), Guaicol (Sisco Research laboratory Pvt. Ltd. Andheri-Mumbai India), Glacial acetic acid, HCl, Methanol, H_2SO_4, Boric acid, Methyl red, Bromocresol, NaOH (HiMedia Laboratories Pvt. Ltd.Vadhani (E) Mumbai India), TPTZ (2,4,6-Tris (2-pyridyl)-s-triazine, ferric chloride, Potassium persulphate, Trolox (6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), DPPH (2,2-Diphenyl-1-picrylhydrazyl), Quercetin, Ascorbic acid, HPO_3, Sodium salt of 2,6- dichlorophenol indophenol dye (Sigma-Aldrich Chemicals Pvt. Ltd. Link road Bangalore, India), ABTS (2,2-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (Roche Diagnostic GmbH Mannheim, Germany), Sodium chloride (Central Drug House (P) Ltd. Daryaganj New Delhi, India) were used in the present study.

3.1.2 Solvent

Ethanol (s. d. fine-chem Limited, Worli, Mumbai, India), Methanol, Petroleum ether (Fisher Scientific India Pvt. Ltd. Powai, Mumbai Indi), Hexane (RFCL Limited Ankhleshwar, Gujarat- India) and Acetone (Fisher Scientific India Pvt. Ltd. Powai, Mumbai India) were used in the present study.

3.1.3 Reagents

3.1.3.1 Acidulants

Tartaric acid, Malic acid, Citric acid and Glucono-Delta-Lactone (GDL) supplied from HiMedia Laboratories Pvt. Ltd. Mumbai, India was used as acidulant.

3.1.3.2 Leavening agent

$NaHCO_3$, calcium carbonate, potassium carbonate and ammonium carbonate supplied from HiMedia Laboratories Pvt. Ltd. Mumbai, India were used as leavening agent.

3.1.3.3 NCDC culture

Lactobacilus ruteri (NCDC- 957) and *Propioniibacterium freudenreichii ssp. Shermanii* (NCDC- 594) were procured from National Collection of Dairy Cultures (NCDC), NDRI, Karnal.

3.1.3.4 Hydrocolloids

Gum acacia or gum Arabic, guar gum, I- carrageenan, xanthan gum, carboxymethyl cellulose (CMC) were supplied from HiMedia Laboratories Pvt. Ltd. Mumbai, India.

3.1.4 Apparatus and glassware

Pipettes, burettes, volumetric flasks, funnels, measuring cylinder (10, 50, 100, 250, 500 and 1000ml), beaker (50, 100, 150 and 250 ml), conical flasks- amber colored and normal (50, 100, 150 and 250ml), volumetric flasks (10,50, 100, 250 and 500 ml), glass test tubes, falcon tubes (amber colored and normal), glass rod, silica crucible, aluminium dishes and aluminium foil were used in the present study.

3.1.5 Grains or Seeds

Grains i.e rice, sorghum and urad dhal were procured from local market of Delhi. These grains were properly packed and stored under dry and cleaned place at room temperature.

3.1.6 *Moringa* pods

Pods were purchased from local market of Karnal, Haryana. The pods were immediately washed, pulped and stored under cold storage temperature of 4°C until use.

3.1.7 Milk

Buffalo milk was procured from Experimental Dairy of the Institute and was used for preparation of Dahi.

3.1.8 Flaked rice

Flaked rice was procured from local market, Karnal

3.1.9 *Idli*

Market *idli*s were obtained from local market Karnal from 10 different restaurants/ hotels and were used for standardization of textural and sensory parameters.

3.1.10 Additives

Common salt was procured from local market, Karnal.

3. 1.11 Equipment

a) Auto pipettes: Themo Scientific, India
b) CIE color system: Hunter lab (Hunter Associates Laboratory), Reston, VA, USA
c) Water bath: The laboratory glassware Co., Timber market, Ambala Cant.
d) Magnetic stirrer: Tarsons, Spinot Model MC 01
e) Texture Analyzer: TAXT2i (Stable micro System, Godalming, Survey, UK)

f) Refrigerator Centrifuge (Sigma 2-16 PK) rotor number: 12071
g) Laboratory centrifuge
h) UV-VIS spectrophotometer: UV-2700, Shimadzu, Japan
i) BOD incubator: Sanco Company
j) pH meter: Eutech, Cyberscan 2100
k) Weighing balance: Citozon, (Range min. 0.01 mg to max 60g)
l) Cabinet tray drier
m) FTIR
n) Rheometer

3.1.12 Microbiological Media

Microbiological media e.g. Potato Dextrose Agar, Violet Red Bile Agar, Plate Count Agar, MRS broth, Trypticase soy broth, Yeast extract, Sodium lactate syrup were obtained from HiMedia Laboratories Pvt. Ltd. Mumbai, India.

3.2. Methods

3.2.1. *Moringa* pulp powder preparation and storage

Moringa pods were washed with potable water until it became debris or soil-free. These were then cut into pieces of length of 5-6 cm for blanching purpose. Blanching was done using steam and was ensured by peroxidase inactivation test which is most heat resistant enzyme. After blanching, pulp and seeds were removed/ extracted from pod's hardest outer fiber portion. The pulp with seeds were grounded by using mixer. Ground *Moringa* pulp with seed was dried in cabinet tray drier, dried material was ground into powder using mixer and stored in an air tight container until further use.

3.2.1.1. Peroxidase test

Peroxidase test was done by using procedure of ISI (1984) with slight modifications. Five grams sample of blanched *Moringa* pulp with seed were weighed and mashed with 10ml of distilled water, then filtered through bloating paper, 2ml of filtrate was taken into test tube followed by addition of 1ml of 1% guaiacol in alcohol, then addition of 1ml of 0.3% of H_2O_2 solution was done. Test tube solution was mixed quickly and absence of red ring/ red colour indicated complete inactivation of peroxidase enzyme.

3.2.2 Optimization of processing steps and ingredients for development of functional *idli* mix

Several different processing techniques were adopted like natural fermentation, dry and wet pre-gelatinized methods, use of yeast and bacterial culture for instant fermentation, etc., for process optimization to obtain final product which is similar to *idli* obtained by natural fermentation but require minimum preparation steps. The various approaches adopted for preparation of instant *idli* mix are described in subsequent subsections and given in Fig. 3.1. The results pertaining to the development of end product are described in Chapter 4.

3.2.2.1 Pre-gelatinization

The different methods of pre-gelatinization were employed in the present study and their effect on the quality attributes of *idli* were analyzed sensorially.

3.2.2.1.1 Dry roasting

Pre-gelatinization was done by dry cooking. For this, grains were ground and dry roasting was done with little quantity of ghee for 2 minutes.

3.2.2.1.2 Wet pre-gelatinization (hydrothermal processing)

Grains were washed and soaked for different time period i.e. ½, 1, 1 ½, 2, 2 ½, 3, 3 ½ and 4 hours with different ratios of water for each grain i.e. 1:1.5 (Rice: water), 1:2 (Urad dhal: water) and 1:1.5 (Sorghum: water). Optimization of soaking time was done based on increased weight of grains at different period of time which was tested statistically. The soaked grains were steam parboiling as per the method and timings given by Gala'n and Drago (2018) i.e. sorghum and urad dhal were steam parboiled in boiling water for 8 minutes and 10 minutes, respectively.

3.2.2.2 Optimization of fermentation time of grains

Grains were washed with potable water until grains became debris and soil free. Then grains were soaked in potable water at different ratios with water which was selected based on the preliminary analysis. The time of soaking varied for 8 different time period. After the fermentation of grains, these were dried in cabinet tray. Dried grains were ground and moisture content was noted down at every 0.5 hours interval. The drying curve was prepared with moisture of sample versus time and rate of moisture removed versus time. The grains were then stored in air tight container until further use. The *idli* prepared from these combinations were evaluated sensorially for optimization of fermentation time of grains and selected time was used for study further.

3.2.2.3 Fermentation of *idli* batter using specific cultures

The approach of fermentation of *idli* batter using specific cultures were also adopted. For this, first activation of culture was carried out. Sodium lactate broth (for *Propioniibacterium freudenreichii ssp. Shermanii*) was prepared using 1% trypticase soy broth, 1% yeast extract, 1% sodium lactate syrup and MRS broth (for *Lactobacillus ruteri*) was prepared using 5.54 g of MRS

Plate 3.1 Microscopic image of *Bacillus* and activated *Lactobacillus* culture in broth

Plate 3.2 Activated *Propionic acid bacteria* culture in MRS broth

broth in 100 ml. Ten milliliters of broth tubes of both were prepared and sterilized. Those tubes were kept in incubator for overnight at 37°C to find out growth of unwanted microbe. Freeze dried culture was inoculated in sterilized sodium lactate broth. The inoculated broth was incubated for 16 hours at 37°C. Sub-culturing was done in 12% skimmed milk. The activated cultures were used in the batter for fermentation purpose as indicated in Chapter 4.

3.2.3 Mix Preparation

After selection of the processing steps, the ingredients for development of functional *idli* mix were optimized using 2^5 factorial experiment as indicated in Table 3.1. For this, the different

levels of RE, UD, SR, MPP and CR (coding is done and followed in entire thesis for IPR issues) have been utilized with different adjuncts like hydrocolloids, flavor enhancer, etc.

The optimized formulation was further selected for development of end product which along with the control samples was analyzed for parameters as indicated in subsequent sections.

3.3 Analytical methods

3.3.1 Compositional analysis

3.3.1.1 Estimation of Protein content

The protein content of functional *idli* mix and control were determined by Macro Kjeldahl method employing digestion, distillation and titration.

***Digestion*:** In a clean and dry Kjeldhal flask (500 mL), 5-10 boiling aids and nitrogen free 3g digestion mixer having potassium sulphate and copper sulfate in 9:1 ratio were added. 5±0.1g *idli* mix was taken (after accurate weighing to the nearest 0.1 mg). Potassium sulphate or sample residues remaining on the neck of the flask were washed down by the addition of 25 mL of concentrated H_2SO_4 (Strength: 95-98% m/m; nitrogen free; density approximately 1.84 g/mL). This mixture was then gently mixed followed by digestion at 420°C temperature till clear solution (without any black specs) was obtained.

***Distillation*:** After complete digestion, digested sample was cooled to room temperature followed by making up the volume to 100 mL with distilled water in the volumetric flask. The whole content was thoroughly mixed to ensure complete dissolving of any crystals and cooled to room temperature. About 10mL of digested content was taken and poured into Kjeldhal flask. Immediately after this, the flask was connected to the distillation apparatus, the tip of the outlet on heating mettle to continue the distillation. A total of 200 mL of the distillate was, thus, collected in nitrogen free conical flask.

***Titration*:** The distillate (200mL) was then removed from distillation assembly and titrated against 0.1N hydrochloric acid. The appearance of slight violet color indicated the end point. The titer volume was noted. One reading for blank was initially taken by replacing *idli* mix sample with 5 mL water. The protein content was calculated as follows:

$$Nitrogen\ content\ (\%) = (1.4007 \times (VS - VB) \times \mathrm{N}) / Weight\ of\ sample\ (W)$$

$$\text{Protein (\%)} = \text{\% Nitrogen} \times \mathrm{F}$$

where, VS = Volume in mL of the standard hydrochloric acid used for sample

VB = Volume in mL of the standard hydrochloric acid used for blank

N = Normality of HCl (0.1N); W = Mass of test portion in g, expressed to nearest 0.1 mg

F = Conversion factor for nitrogen to protein (= 6.25)

Table 3.1 Different combinations of ingredients as per factorial design used for preparation of functional multi-grain *Idli* Mix

Treatments	RE (parts)	UD (parts)	SR (parts)	MPP (parts)	CR (g)
T1	P	R	T	V	X
T2	P	R	T	W	X
T3	P	S	T	V	X
T4	P	S	T	W	X
T5	P	R	U	V	X
T6	P	R	U	W	X
T7	P	S	U	V	X
T8	P	S	U	W	X
T9	Q	R	T	V	X
T10	Q	R	T	W	X
T11	Q	S	T	V	X
T12	Q	S	T	W	X
T13	Q	R	U	V	X
T14	Q	R	U	W	X
T15	Q	S	U	V	X
T16	Q	S	U	W	X
T17	P	R	T	V	Y
T18	P	R	T	W	Y
T19	P	S	T	V	Y
T20	P	S	T	W	Y
T21	P	R	U	V	Y
T22	P	R	U	W	Y
T23	P	S	U	V	Y
T24	P	S	U	W	Y
T25	Q	R	T	V	Y
T26	Q	R	T	W	Y
T27	Q	S	T	V	Y
T28	Q	S	T	W	Y
T29	Q	R	U	V	Y
T30	Q	R	U	W	Y
T31	Q	S	U	V	Y
T32	Q	S	U	W	Y

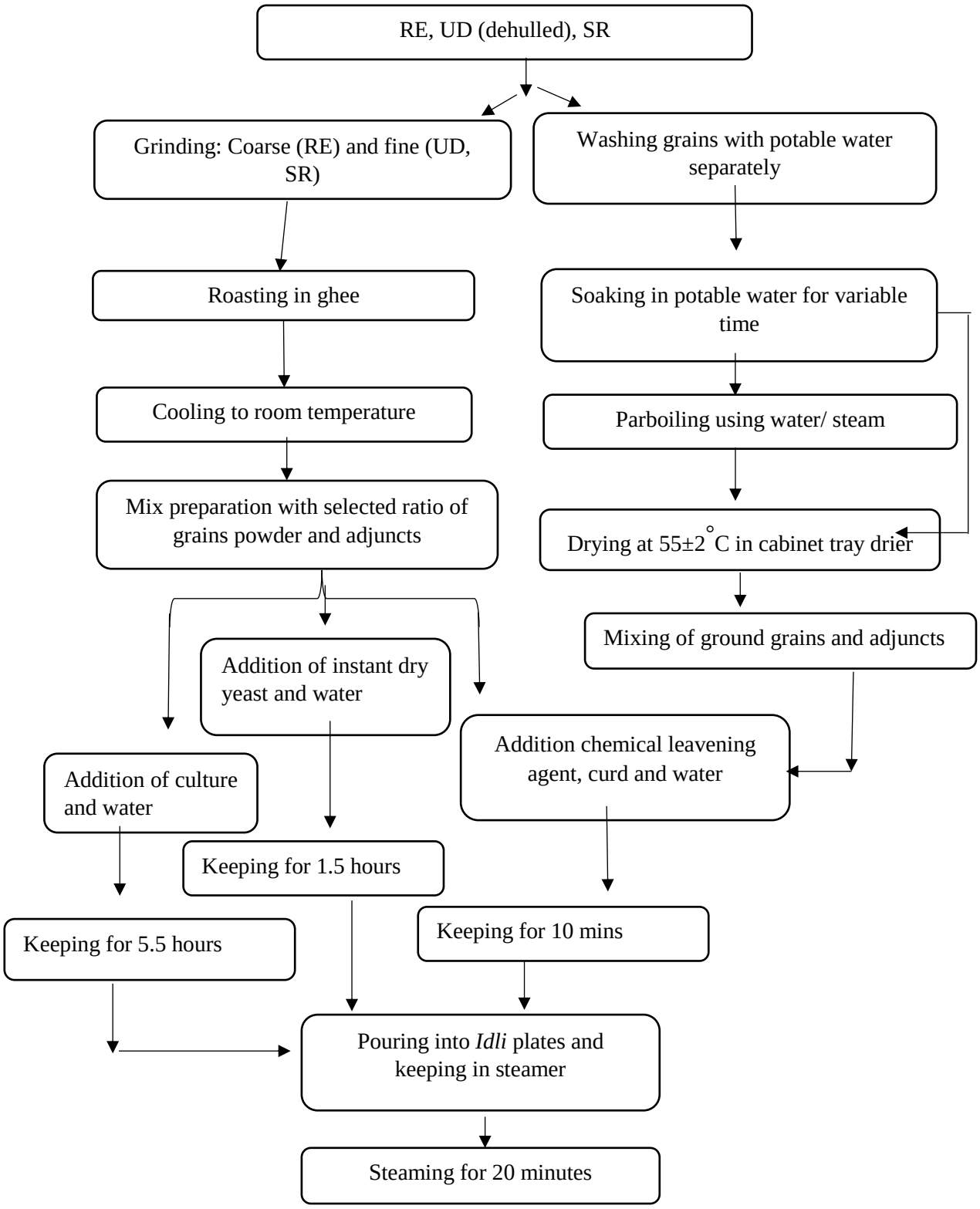


Fig 3.1 Approaches adopted for preparation of *idli* mix

3.3.1.2 Estimation of Fat

Crude fat in grains and *idli* mix was determined by Soxhlet method (AOAC, 2006). Ten grams of dried moisture free grain powder and *idli* mix were taken and put in to thimble. The thimbles were placed inside the soxhlet extractor and was joined with round bottom flask. The flask was placed on the heating mantle. Petroleum ether (250ml for each sample) was poured into soxhlet extractor through condenser mouth open. Then soxhlet inlet, outlet and heating mantle were switched on. The temperature was kept at 60^{O}C as is the boiling point of petroleum ether. After complete fat extraction, the petroleum ether was first evaporated on the water bath followed by evaporation of petroleum ether in hot air oven at around 100°C for complete evaporation. Then, fat was calculated using following formula

$$Fat\ \% = \left(\frac{M3 - M2}{M1}\right) X\ 100$$

M1- Weight of sample

M2- Weight of empty round bottom flask

M3- Weight of fat and round bottom flask

3.3.1.3 Estimation of Moisture

Moisture content of grains and mix were analyzed by method given by Ranganna (1986). About 5g of sample was taken and placed in moisture dishes and kept in hot air oven for 3 hour or till the constant weight was achieved. After drying, the samples were taken and then cooled in desiccator to attain room temperature. The data were recorded and the moisture content was calculated using the following formula:

$$\text{Moisture\%} = \frac{\text{W2} - \text{W3}}{W2 - W1} X\ 100$$

where,

W1: weight of empty dish

W2: weight of dish with sample before drying

W3: weight of dish with sample after drying

3.3.1.4 Estimation of Total ash

The ash content of grains and *idli* mix was determined gravimetrically as per the method of Ranganna (1986) with slight modifications. About 10 g of sample was taken in pre-weighed silica crucible and incinerated on a heater till smoke free. The contents of the crucible were ignited

in a muffle furnace at a temperature not more than 550°C for 6 hours until the ash was free from carbon. The residue was cooled in a desiccator and weighed. The ash content was calculated as follows:

$$Ash\ (\%) = (Weight\ of\ residue\ /Weight\ of\ sample) \times 100$$

3.3.2 Physico-chemical attributes

3.3.2.1 Estimation of pH

About 10 g of *idli* batter was taken for pH determination at 20°C. The pH electrodes assembly was calibrated with standard buffers of pH 7.0, 4.0 and 9.0 before pH estimation

3.3.2.2 Estimation of Titratable acidity

Acidity was determined by following A.O.A.C (1970) with slight modifications. Ten grams of batter in 10ml distilled water was taken, which was mixed well and then 2-3 drops of phenolphthalein indicator was added. For mix, 10g of mix was mixed with 100ml distilled water, which was filtered and form that filtrate 10ml was taken. To the filtrate, 2-3 drops of phenolphthalein indicator were added and which was titrated against 0.1N NaOH till light pink color was observed which persisted for about 15 s.

$$Acidity = 9*A*N \div W$$

Where,

A- Volume of NaOH used for titration

N-Normality of NaOH

W-Volume of sample

3.3.2.3 Estimation of Colour value

Tristimulus spectrophotometer Hunter Lab Colour Flex was used to measure the color of the *idli* mix samples. The results were expressed in terms of CIELAB system. The instrument was standardized in day light at reflectance angle of 10^o (i.e., illuminant D65/10^o standard observer). Before the test, the instrument was calibrated with standard black and white tiles as specified by the manufacturer (i.e., L* 50.83, a* -26.27 and b* 12.12). The light source was dual beam flash lamp. Measurements were then made on the sample taken in a glass sample cup (10 cm height and 6 cm diameter) supplied with the instrument by filing it to a fixed level (up to 3 cm) for each sample. Data was received through the software in terms of L* (lightness), ranging from (0) black to (100) white, a* (redness), ranging from +60 (red) to -60 (green), and b* (yellowness), ranging from +60 (yellow) to -60 (blue) values. During color measurement, care was taken to avoid

breaking of the sampling cup. Three random readings per sample of color were recorded and average was taken.

3.3.2.4 Estimation of internal structure of *Idli* by Ink print test

Number of pores were determined as per the method given by Nazni and Shalini (2010). *Idli* was cut into two equal parts and pressed on stamp pad for inking it, the stamping was done on graph sheet. The pores present per square were counted which was used to denote softness of *idli*. The higher number of pores indicated higher softness in the product.

3.3.3 Bio-functional/ nutritional attributes

3.3.3.1 Estimation of Antioxidant activity

3.3.3.1.1 ABTS (2,2-Azino-bis- 3-ethylbenzothiazoline-6-sulphonic acid) antioxidant activity

Preparation of ABTS reagent

The ABTS antioxidant activity was measured by the method given by Awika *et al.,* (2003). Seven millimolar ABTS solution and 2.45 mM potassium persulphate solution were mixed in equal proportions and allowed to react overnight (16 hours) in dark to obtain ABTS free radicals. Thereafter, the working solution was prepared by diluting with phosphate buffer (pH 7.4, 150mM NaCl) solution to obtain absorbance of 0.70 ± 0.05 at 734 nm.

Sample preparation

The extraction of phenolics was done by using the method reported by Moore *et al.,* (2006). Conc HCl was added in 15ml of 100% methanol at the rate of 1%. 15 ml of this was added into 10g of sample and were kept for stirring for 30 minutes and centrifugation was done for 20 minutes at 4000 rpm at 5°C. The supernatant was collected separately. Then, 15 ml of 80% methanol containing 1% HCl was added into the residue. The centrifugation was done at 4000rpm at 5°C for 20 minutes and supernatant was collected along with previously collected supernatant. Again 15 ml of 50% methanol containing 1% HCl was added followed by centrifugation for 20 minutes at 4000 rpm at 5°C. Supernatant was collected with previously collected supernatant and stored at -20^{O}C till further use. This sample was utilized for total phenolics analysis, flavonoid analysis and determination of anti-oxidant activity.

Hundred µl of sample extract was mixed with 2900 µl ABTS working solution and allowed to react in dark. The absorbance was measured at 734 nm after 10 min. Trolox was taken as standard antioxidant and absorbance was obtained according to same procedure as that for sample. The standard curve (Fig 3.2) was prepared using Trolox standard solutions (µg/ml). The

results of the sample were expressed in terms of TEAC i.e., Trolox equivalent antioxidant capacity (mg/g).

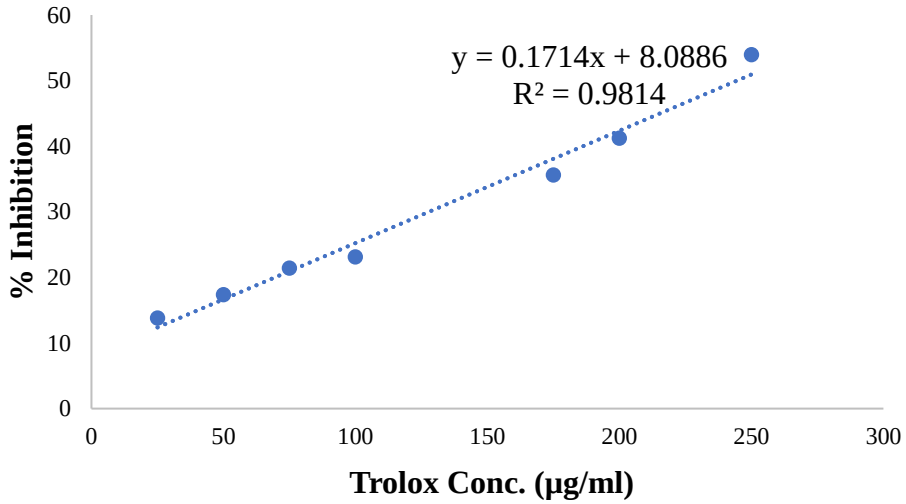


Fig 3.2 Standard Curve for estimation of ABTS

3.3.3.1.2 DPPH (2,2- Diphenyl-1-picrylhydrazyl) antioxidant activity

DPPH antioxidant activity of the *idli* mix was measured according to the method given by Cuendet *et al.*, (1997) with some modifications. The main principle i.e. DPPH radical was reduced by reaction with antioxidant resulting in reduction of the absorbance. For analysis, 50 µl sample extract was mixed with 3.95 ml of methanol and reaction was carried out by adding 1 ml of 0.1M DPPH (DPPH in 80% methanol was stirred for overnight). After 30 min of incubation in dark at room temperature, the absorbance was measured at 517 nm using methanol as blank.

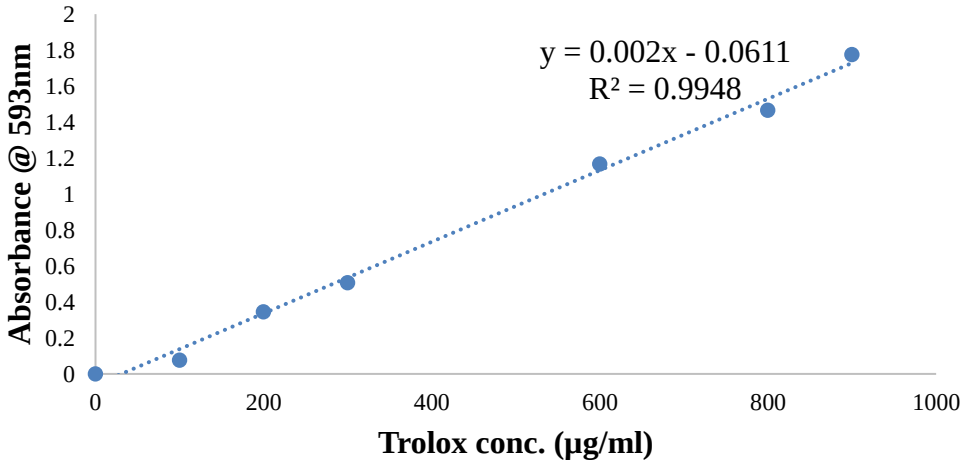


Fig 3.3 Standard Curve for estimation of DPPH

Standard curve (Fig 3.3) was prepared using trolox (µg/ml) as standard. Results were expressed in terms of DPPH scavenging activity (%).

$$Antixidant\ activity = \frac{Control\ absorbence - sample\ absorbence}{Control\ absorbence} * 100$$

3.3.3.1.3 FRAP (Ferric Reducing Antioxidant Power) antioxidant activity

The FRAP antioxidant activity was analyzed according to the method given by Benzie and Strain (1996) with little modifications. Three hundred millimolar acetate buffer (pH 3.6 prepared by adding 0.31g sodium acetate trihydrate in 1.6mL glacial acetic acid and making up the volume to 100mL by distilled water), 10 mM TPTZ (prepared by adding 0.1561g TPTZ in 50ml of 40 mM

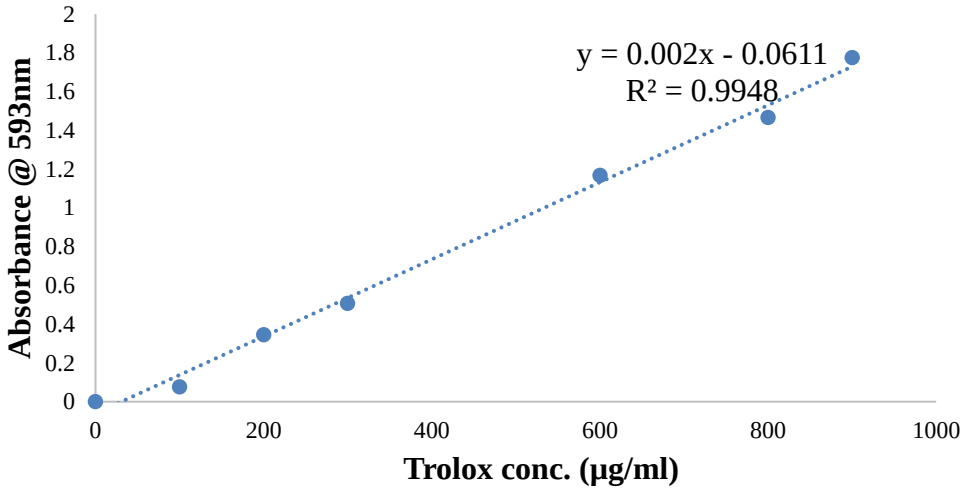


Fig 3.4 Standard Curve for estimation of FRAP

HCl solution) and 20 mM ferric chloride solution (prepared by adding 0.2703g of ferric chloride in 50mL distilled water) were prepared. Sample preparation was done as mentioned in section 3.3.3.1.1. 2850 µL of FRAP reagent (prepared by mixing 30 mL of the acetate buffer, 3 mL of the TPTZ solution and 3 mL of ferric chloride solution) was mixed with 150µL of the sample. The prepared FRAP reagent was warmed to 37°C before use. The absorbance was then measured at 593 nm using spectrophotometer after exactly 30 min of incubation. Standard curve (Fig 3.4) of trolox was prepared at six concentrations (0, 20, 40, 60, 80, 100, 120, 140 and 160 µM).

3.3.3.2 Estimation of Total Phenolic

The total phenolic content of *idli* mixes were determined by Prussian blue method given by Graham (1992). Three ml of sample extract was taken in which 1ml of 0.016M Potassium

ferricyanide ($K_3[Fe(CN)_6$) was added followed by addition of 0.02M of Ferric chloride dissolved in 0.1M HCl. After mixing, these were incubated at 24±1°C for 15 mins in dark condition.

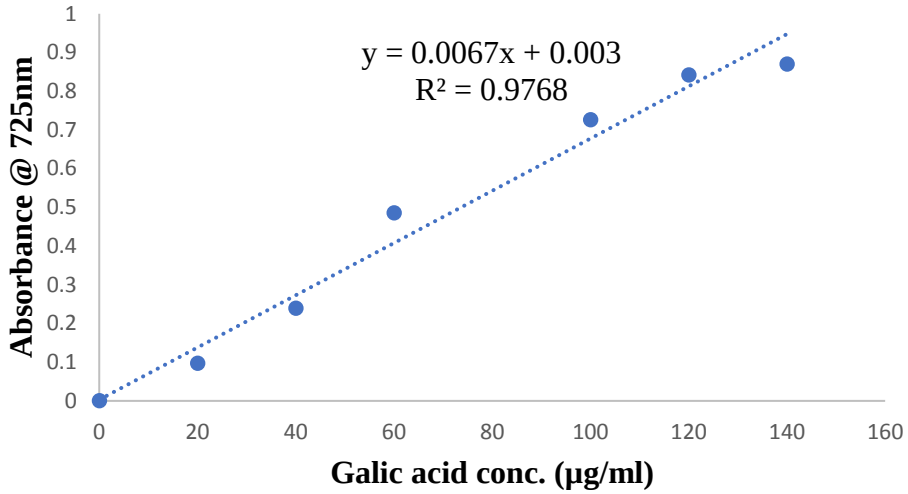


Fig 3.5 Standard curve for estimation of total phenolics content

The absorbance was then was recorded at λ=725 nm. The standard curve (Fig 3.5) was prepared with standard gallic acid with procedure as applied for sample. The total phenolic content was expressed as µg gallic acid eq./ g of sample.

3.3.3.3 Estimation of Flavonoid

The flavonoids of *idli* mix were analyzed using the method given by Zhuang *et al.*, (1992) with little modification. Two milliliter of sample extract was taken and diluted with methanol to 5 ml. It was followed by addition of 0.3ml of 5% sodium nitrate and kept aside for 5 minutes. Further, 0.3ml of 10% aluminium chloride was added into it and kept for incubation for 6 minutes. Then, 1ml of 1M NaOH was added and the volume was made up to 10 ml with methanol. The solution was kept for ½ hours of incubation and absorbance was taken at 510 nm using methanol as blank. The standard curve was prepared using quercetin (µg/ml) as standard and flavonoid content was expressed as quercetin equivalent per gram of sample (Fig 3.6).

3.3.3.4 Estimation of Total carotenoid

The estimation of total carotenoid content was done as per the protocol proposed by Luterotti and Kljak (2010) with some modifications. One g or ml (depending upon the nature of

sample) of sample was accurately weighed and was mixed with 10 ml, 75% of acetone in amber colored conical flask and was mixed on magnetic stirring for 30 min. The addition of 2.5 ml of

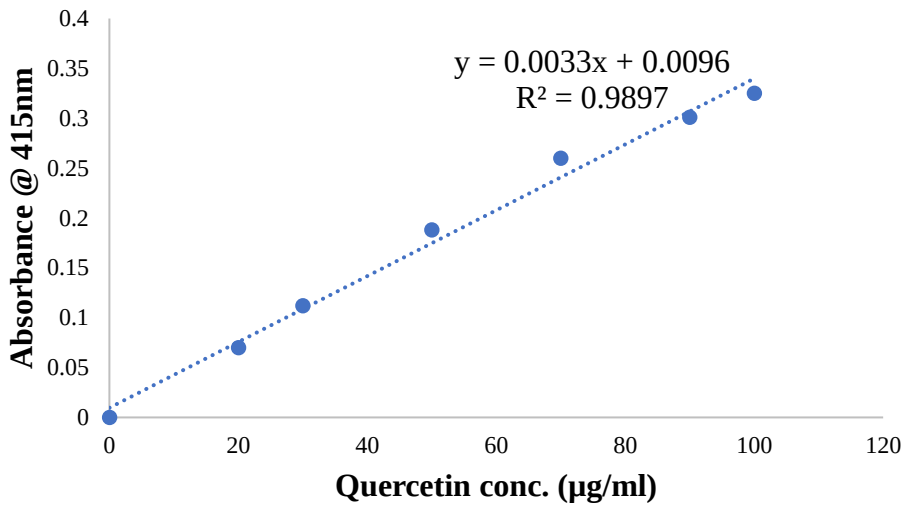


Fig 3.6 Standard Curve for estimation of Flavonoids content

hexane was done into it and the mixture was stirred for 30 min. This was followed by addition of 15 ml of solvent mixture (Hexane: Acetone: Ethanol in ratio of 2:1:1) and subjected to stirring for 30 min followed by addition of 2.5 ml of distilled water with shaking for 5 min. The mixture was subjected to centrifugation @ 4000 rpm for 5 min and upper layer of hexane was quantitatively removed. In second extraction step, 15 ml of hexane was added and extraction was carried out as described above. The total volume of hexane obtained was used for the calculation of total carotenoids content. The absorbance of the hexane layer was analyzed at 450 nm using pure hexane as blank in spectrophotometer. The total carotenoids (µg / g) in the sample was calculated using following formula.

$$\text{Total carotenoids content (µg / g or µg /ml)} = \frac{A*V*10000}{\varepsilon*P}$$

where,

A= Absorbance at 450 nm

V= Total Volume of hexane layer (ml)

ε (A1% 1cm) = Extinction co-efficient of total carotenoids in hexane (2500 dL/g cm)

P= Weight (g) or Volume (ml) of sample

3.3.3.5 Estimation of vitamin C

Ascorbic acid or vitamin C was analyzed by the method of Ranganna (1986) with slight modifications.

Sample preparation

Ten grams of *idli* mix was blended with 3% HPO_3 and made up to 100 ml in volumetric flask with HPO_3, then filtration and centrifugation was done at 2500 rpm for 10 minutes. Supernatant was taken out separately.

Dye preparation

Fifty milligrams of sodium salt of 2,6- dichlorophenol indophenol was dissolved in 150ml of hot distilled water containing 42 mg of $NaHCO_3$. It was cooled down and diluted with distilled water to 200ml and was stored under refrigerated storage and standardized every day until used.

Standardization

Five milliliter of standard ascorbic acid (100 mg of L-ascorbic acid was taken and made up to 100 ml with 3% HPO_3). Ten ml of stock solution was diluted with 100ml of 3% HPO_3, which was expressed as 1ml= 0.1mg of ascorbic acid of standard and was used as working standard. Five ml from working standard was taken along with 5 ml of 3% HPO_3, which was titrated against dye until pink colour persisted for 15 s as end point. The dye factor was then calculated using following formula:

$$Dye\ factor = \frac{0.5}{titre\ value}$$

Assay of extract

Ten ml of extract was titrated against standard dye. The end point was pink in colour which persisted for 15 s. Ascorbic acid was calculated using formula:

mg of ascorbic acid/100g of sample=

$$\frac{\text{Titre} * \text{Dye factor} * \text{volume made up} * 100}{\text{Aliquot of extract taken for estimation} * \text{weight sample taken for estimation}}$$

3.3.3.6 Estimation of Iron

3.3.3.6.1 Ash solution preparation

Forty ml of 1:1 diluted HCl was added into dried ash in crucible and which was heated for 30 minutes until HCl in crucible dehydrated completely. Then, another 10 ml of diluted 1:1 HCl was added into crucible for rinsing purpose. This solution was filtered through Whatman No. 40 followed by washing crucible and filter paper with water. Finally, filtered solution was made up to 100ml in volumetric flask. This solution was used for calcium and iron analysis as mineral or ash solution.

3.3.3.6.2 Standard and sample preparation for iron estimation

Iron was estimated by following the method of Ranganna (1986) with slight modifications. Standard was prepared with 0, 0.5, 1, 1.5, 2, and 2.5 ml of standard iron solution (0.0702 g of ferrous ammonium sulphate was dissolved in 10ml of distilled water, 5ml concentrated H_2SO_4 was added and slightly warmed, and conc. $KMnO_4$ was added drop by drop for the formation of permanent color and volume was made up to 100ml) was taken in to different test tubes. 0.5 ml of conc. H_2SO_4, 1ml of potassium persulphate (0.7 to 0.8 g in 10ml of distilled water) and 2ml of potassium thiocyanate (14.6 g in 50ml distilled water and with addition of 2ml acetone to maintain quality) were subsequently added. Then, the volume was made up to 15 ml with distilled water. The absorbance was taken at 480nm immediately after making up the volume. The sample was prepared like above mentioned procedure but instead of standard iron solution, 5ml ash solution was taken. Also, blank was prepared without sample and standard iron solution. Standard curve was prepared (Fig 3.7) and iron concentration was calculated.

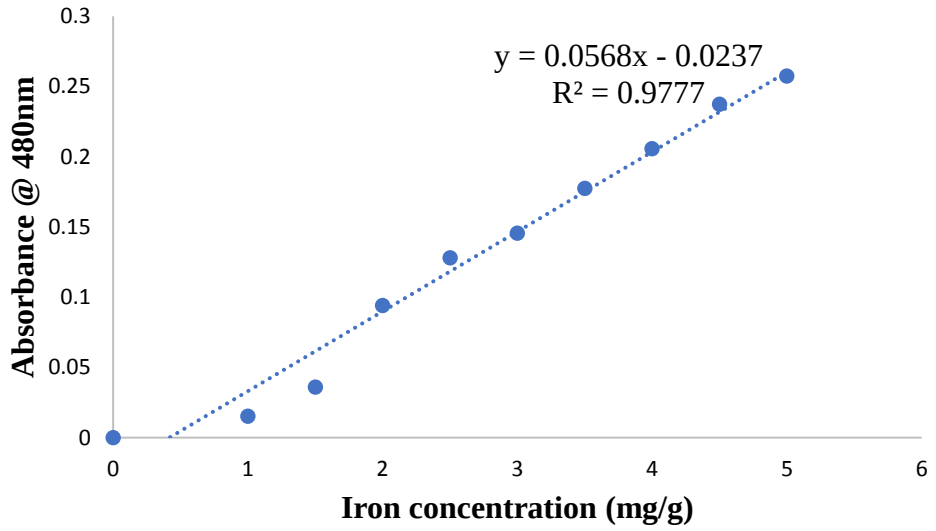


Fig 3.7 Standard curve for estimation of iron content

3.3.3.7 Estimation of Calcium

Calcium in samples was determined by following the method of Ranganna (1983) with slight modifications. Twenty milliliters of ash solution was taken in a 250ml beaker. Ten milliliters of saturated ammonium oxalate solution and 2 drops of methyl red were added in to ash solution. Then, ammonia was added to make solution alkaline followed by addition of few drops of acetic

acid (until faint pink color formation persisted) to make solution slightly acidic. Then, the solution was heated to its boiling point at room temperature and was kept aside for overnight. The solution was then filtered through Whatman no. 40 filter paper and washing of filter paper was done with distilled water for making filter paper oxalate free. Then, filter paper was broken at center by using glass rod as precipitate form of calcium sedimented on the filter paper, which was washed with hot dil. H_2SO_4 (1+4) in to separate beaker and again the filter paper was washed with hot water. Then, the solution was titrated in hot condition (70-80°C) against 0.01N $KMnO_4$ until pink color formation and then the broken filter paper was added in to the titrated solution. Pink colour of solution was lost by addition of broken filter paper. Again, that filter paper added solution was titrated until the pink color formation. The calcium content was calculated using following formula

$$\boldsymbol{Calcium}\left(\frac{\boldsymbol{mg}}{\boldsymbol{100g}}\right) = \frac{\boldsymbol{titre} * \boldsymbol{0.2} * \boldsymbol{total\ volume\ of\ ash\ solution} * \boldsymbol{100}}{\boldsymbol{Volume\ taken\ for\ analysis} * \boldsymbol{weight\ of\ sample\ for\ ashing}}$$

3.3.4 Texture Profile Analysis

The sample of *idli* was evaluated for its textural attributes using two bite compression test by Texture Analyzer TAXT2i (Stable Micro System, Godalming, Survey, UK) fitted with 25 kg load cell by following protocol of Durgadevi and Shetty (2014) with slight modifications. The cooked *idli* was cooled to room temperature and sample was cut into 1 x 1 x 1 cm^3**.** This sample piece was placed on the base plate of texture analyzer and 50% strain was applied with P75 probe to find textural attributes. Values were taken in triplicate for each sample.

Table 3.2 Test condition for TPA

Conditions	**Range**
Pre-test speed	2mm/s
Test speed	5mm/s
Post-test speed	2mm/s
Distance	15mm
Strain	50%
Resting gap	2s
Load cell	25kg

3.3.4.1 Hardness

Hardness is represented by the first peak in the graph or maximum force required at first compression. It is expressed in Newtons.

3.3.4.2 Adhesiveness

Adhesiveness is represented by the negative peak in the graph. It is expressed in N·s

3.3.4.3 Springiness

Springiness is expressed as ratio or percentage of products height. It is percentage of detected height during the second compression divided by the original compression distance. Generally, springiness is how well a product physically springs back after it has been deformed during the first compression.

3.3.4.4 Cohesiveness

The area of work during the second compression divided by the area of work during the first compression is called cohesiveness. Cohesiveness is how well the product withstands a second deformation relative to its resistance under the first compression.

3.3.4.5 Chewiness

Chewiness is defined as the product of hardness x cohesiveness x springiness. Expressed in Newtons.

3.3.4.6 Resilience

Resilience is how well a product fights to regain its original height. It is calculated on the withdrawal of the first penetration, before waiting period is started.

3.4 Rheological properties of batter from *idli* mix

Batter was analyzed for its rheological parameter like temperature sweep, amplitude sweep, frequency sweep and flow curve in Rheometer (Rheolab, QC Anton Par, USA).

Table 3.3 Test conditions for rheology

Parameters	**Temperature sweep**	**Amplitude sweep**	**frequency sweep**	**Flow cure**
Probe	PP50	PP50	PP50	PP50
Temperature	20-100^{O}C	25^{O}C	25^{O}C	25^{O}C
Rate of heat raising	2^{O}C/s	-	-	-
Gap	1mm	1mm	1mm	1mm
Shear rate	-	-	-	0.01-1000/s
Strain	0.1%	0.01-100%	-	-
Frequency	5 rad/s	5 rad/s	1- 100 rad/s	-

3.5 FTIR spectral analysis

FTIR spectral analysis was done for optimized *idli* mixes. The sample was kept on plate of FTIR and attached to the surface of Dimond crystal cell of attenuated total reflectance (ATR)

crystal having path length of 1.66 μm, where FTIR spectrometer light beam was passed. The *idli* mix sample was analysed. Total 40 scans were taken for absorption for each sample in the wavenumber range from 4000 cm^{-1} to 400 cm^{-1}. The Fourier transform infrared (FTIR) spectrometer sample absorption spectra was taken at resolution of 4 cm^{-1} and scan speed of 0.2 $cm \cdot s^{-1}$. The background spectra check was recorded before introducing sample to the blank ATR crystal. The ATR crystal was cleaned every time before acquiring the next sample with soft tissue impregnated with isopropanol followed by drying of crystal. The FTIR data of *idli* mixes were collected in OPUS software (7.2 Build, Bruker Optik GmbH).

3.6 Microbial Analysis

The optimized *idli* mix was examined for the total plate count, yeast and mold count and coliform count.

3.6.1 Preparation of dilution

Exactly 11g of sample was added into 99 ml of sterile dilution blank. The content of the flask were mixed well. This represented the first dilution (1:10), subsequent dilutions were prepared by transferring 1mL of the sample solution in 9mL sterile dilution blanks.

3.6. 2 Total plate count

The total number of viable bacteria in *idli* mix was enumerated by the method described by Houghtby *et al.,* (1992) using plate count agar (pH 7.0±0.1) as nutrient medium. The prepared plates were incubated at 37°C for 48h.

3.6.3 Yeast and mold count

The yeast and mold counts were enumerated by method described by Marshall (1993) using potato dextrose agar (pH 3.5±0.1). The prepared plates were incubated at 25°C for 3-5 days and counts were expressed as log cfu/mL of sample.

3.6.4 Coliform count

The coliform counts were enumerated by method described by Houghtby *et al.,* (1992) using violet red bile agar (pH 7.4±0.1). The prepared plates were incubated at 37°C for 48h and counts were expressed as coliforms per g of sample.

3.7 Sensory analysis

The *idli* prepared form *idli* mix was evaluated by Descriptive analysis score card for describing the sensory attributes of *idli* like colour, surface appearance, shape, dryness of interior, porosity, firmness, stickiness, springiness, fermented aroma, acidic taste, stale and overall

acceptability by trained judges selected from the faculty of Dairy Technology Division, NDRI, Karnal. The sensory score is attached as Annexure.

3.8 Statistical analysis

All the experiments were carried out in triplicate. Results are expressed as mean ± standard error (SE). The data obtained during optimization were analyzed statistically for sensory, texture profile analysis and ink print text by using SPSS 23 with 2^5 factorial experiment of completely randomized design and proximate composition, physico-chemical attributes, bio-functional attributes, rheological properties, texture profile parameters, sensory analysis and microbial analysis of optimized product and control were analyzed for significant different using one way ANOVA and Tukey's range test ($p<0.05$).

CHAPTER 4

Results and Discussion

The present study was carried out with the objective of developing a functional *idli* mix using *Moringa* pods with nutri-cereal i.e., sorghum as an important ingredient. The work was carried out systematically in two phases and was further divided into four sub-phases. The sub-phases of first phase included (a) texture profile analysis and descriptive sensory analysis of market sample for standardization of parameters to be used for preparation of final product i.e. functional multi-grains *idli* mix (b) optimization of the steps for preparation of final product (c) optimization of ingredients (RE, UD, SR, MPP and CR) for preparation of functional multi-grain instant *idli* mix (FMIIDM) (d) preparation of optimized product along with that of the control *idli* mix (CM) and control *idli* (CID). The second phase of investigation involved analysis of physico-chemical, bio-functional, rheology, sensorial and microbiological parameters of optimized and control *idli* mix/ batter/ *idli* prepared from these mixes.

4.1 Texture profile analysis and Descriptive sensory analysis of market sample for standardization of parameters to be used for optimization of functional multi-grain instant *idli* mix (FMIIDM)

Idli is cereal (rice) and legumes (black gram or urad dal) based steamed or leavened cake made from fermented batter. It is a popular product of India and Sri Lanka (Durgadevi and Shetty, 2012). Generally, *idli* is white in colour, disc in shape, soft and spongy in texture (Agarwal *et al.*, 2000), sour in taste with peculiar fermented aroma due to volatile and non-volatile compounds like ketones, diols, alcohol and acids produced by microbes in the batter during fermentation (Agarwal *et al.*, 2000). Market *idli* samples were procured from 10 different hotels of Karnal and were evaluated for descriptive sensory analysis, texture profile analysis and ink print test. The descriptive sensory score card was developed for the research work and included parameters of color and appearance (colour, surface area and shape); appearance of cross section (dryness of interior and porosity); texture (firmness, springiness); flavour (fermented aroma, acidity taste and stale) and overall acceptability. The texture profile analysis of the *idli* samples was carried out for parameters like hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience as these are of utmost importance and correlate well with the sensory attributes. In past, Durgadevi and Shetty (2012) have also used some of these parameters (colour, fluffiness, sponginess, fermented aroma, compactness, firmness, sourness and overall acceptability) for studying sensorial attributes of *idli*. The parameters were analyzed statistically using one-way ANOVA with

Table 4.1 Descriptive sensory scores of markets *idli* samples

Market	Colour	Surface appearance	Shape	Dryness interior	Porosity	Firmness	Springiness	Fermented aroma	Acidic taste	Stale	Over all acceptability
1	4.17 ^{a}±0.73	6.57 ^{a}±0.67	5.00 ^{a}±0.45	6.50 ^{a}±0.52	7.00 ^{a}±0.55	3.67^{ab}±0.60	3.73 ^{a}±0.72	7.87 ^{c}±0.79	7.90^{b}±0.84	0.17 ^{a}±0.05	6.03 ^{a}±0.51
2	3.50 ^{a}±0.77	6.67 ^{a}±0.50	6.23 ^{a}±0.65	5.17 ^{a}±0.33	7.00 ^{a}±0.60	3.57^{ab}±0.37	5.23 ^{a}±0.38	6.47^{bc}±0.80	4.07^{ab}±0.80	0.40 ^{a}±0.12	7.33 ^{a}±0.37
3	7.37 ^{a}±0.79	5.73 ^{a}±0.53	7.37 ^{a}±0.45	5.00 ^{a}±0.48	4.60 ^{a}±0.65	5.17^{abc}±0.56	6.57 ^{a}±0.62	6.53^{bc}±0.86	4.17^{ab}±1.19	0.07 ^{a}±0.75	7.77 ^{a}±0.69
4	4.83 ^{a}±0.42	6.60 ^{a}±0.73	6.10 ^{a}±0.74	6.50 ^{a}±0.57	5.93 ^{a}±0.50	5.40^{abc}±0.44	7.03 ^{a}±0.66	4.50^{abc}±0.75	1.77^{ab}±1.02	0.17 ^{a}±0.25	5.87 ^{a}±0.70
5	7.20 ^{a}±0.71	7.73 ^{a}±0.79	6.63 ^{a}±0.65	5.63 ^{a}±0.47	4.90 ^{a}±0.54	3.50^{ab}±0.34	5.00 ^{a}±0.35	8.37^{c}±0.79	6.73^{ab}±1.16	0.00 ^{a}±0.65	7.63 ^{a}±0.29
6	5.20 ^{a}±0.64	4.30 ^{a}±0.70	6.13 ^{a}±0.71	5.17 ^{a}±0.42	6.77 ^{a}±0.37	3.37^{a}±0.37	7.00 ^{a}±0.49	1.00^{a}±1.04	1.37^{a}±0.61	2.17 ^{a}±0.33	5.17 ^{a}±0.59
7	2.50 ^{a}±0.32	4.53 ^{a}±0.58	7.80 ^{a}±0.61	5.50 ^{a}±0.67	5.03 ^{a}±0.55	7.20^{c}±0.25	3.47 ^{a}±0.73	2.10^{ab}±0.91	2.93^{ab}±0.62	0.83 ^{a}±0.33	3.73 ^{a}±0.64
8	5.97 ^{a}±0.64	4.00 ^{a}±0.42	5.00 ^{a}±0.43	4.23 ^{a}±0.54	6.17 ^{a}±0.50	5.00^{abc}±0.18	4.17 ^{a}±0.70	5.80^{abc}±0.73	3.43^{ab}±0.50	1.00 ^{a}±0.57	5.97 ^{a}±0.65
9	5.30 ^{a}±0.87	5.67 ^{a}±0.54	6.67 ^{a}±0.66	5.83 ^{a}±0.40	6.17 ^{a}±0.58	2.83^{a}±0.37	4.00 ^{a}±0.86	5.83^{abc}±0.68	4.17^{ab}±0.60	0.67 ^{a}±0.51	7.33 ^{a}±0.67
10	5.10 ^{a}±0.78	4.83 ^{a}±0.62	5.00 ^{a}±0.60	5.30 ^{a}±0.67	5.00 ^{a}±0.54	6.67^{bc}±0.41	4.73 ^{a}±0.53	7.50^{c}±0.47	6.67^{ab}±0.35	2.00 ^{a}±0.19	5.07 ^{a}±0.73

Mean ±SE, (n=10); Mean values with different superscripts are significantly different with each other ($p<0.05$) within a same column

CD (colour)= 2.06 CD (surface appearance) =1.67 CD (shape)= 1.17 CD (dryness of interior) = 1.72
CD (porosity)=1.88 CD (firmness)=1.06 CD (springiness) = 2.33 CD (fermented aroma) =1.59
CD (acidic taste) =1.92 CD (Stale) =1.70 CD (overall acceptability) =2.01

Tukey's test on SPSS 23 for identifying the significant difference between the various market samples with respect to parameters mentioned above. Table 4.1 indicate mean values of descriptive sensory scores of markets *idli* samples as analysed by the sensory panelist, while it can be revealed from Table 4.2 that there existed significant difference ($p<0.05$) between firmness, fermented aroma and acidic taste of different market samples of *idli*. However, all other parameters of sensory evaluation showed non-significant difference ($p>0.05$) between all the market samples. Table 4.3 indicate mean values of parameters of texture profile of market *idli* samples. It can be observed from Table 4.2 and 4.3 that the textural attributes and ink print test showed significant difference ($p<0.05$) between all the 10 different market samples.

Table 4.2 ANOVA for studying sensory parameters, textural properties and Ink Print test of market *idli* samples

Source	Df	Type III SS	MSS	F value	Pr.>F	S/ NS
Colour	9	61.828	6.870	1.406	.250	NS
Surface appearance	9	40.743	4.527	1.412	.248	NS
Shape	9	25.985	2.887	1.832	.124	NS
Dryness interior	9	12.728	1.414	.418	.910	NS
Porosity	9	22.640	2.516	.619	.768	NS
Firmness	9	59.790	6.643	5.186	.001	S
Springiness	9	48.832	5.426	.867	.568	NS
Fermented aroma	9	158.216	17.580	6.044	.000	S
Acidic taste	9	126.628	14.070	3.313	.012	S
Stale	9	16.435	1.826	.551	.820	NS
Overall acceptability	9	47.120	5.236	1.131	.387	NS
Hardness	9	85.624	9.514	37.309	.000	S
Adhesiveness	9	.008	.001	6.249	.000	S
Springiness	9	.029	.003	3.095	.017	S
Cohesiveness	9	.091	.010	3.964	.005	S
Chewiness	9	46.662	5.185	42.580	.000	S
Resilience	9	.014	.002	4.083	.004	S
Ink Print Test	9	16225.467	1802.830	4507.074	.000	S

S-Significant ($p<0.05$); NS- Non-significant ($p>0.05$)

The ranges of values for all the parameters, namely sensory, textural and ink print test are given in Table 4.4. The scores for sensory parameters i.e. color ranged between 2.50 and 7.20, surface appearance ranged between 4.30 and 7.73, shape ranged between 5.00 and 7.8, dryness of

interior ranged between 4.23 and 6.50, porosity ranged between 4.60 and 7.00, firmness ranged between 2.83 and 7.20, springiness ranged between 3.47 and 7.03, fermented aroma ranged between 1.00 and 8.37, acidic taste ranged between 1.37 and 7.90, stale ranged between 0 and 2.00 and overall acceptability ranged between 5.07 and 7.77. The ranges for textural attributes were between ~2.5 N and ~8 N for hardness, ~ -0.5 N.s and 0 N.s for adhesiveness, 0.787mm and 0.884mm for springiness, 0.777 to 0.96 for cohesiveness, 1.706 J and 6.016 J for chewiness, 0.294 and 0.73 for resilience. The number of pores in *idli* ranged between 78 and 145. These values were used in the subsequent study for optimization of FMIIDM.

Table 4.3. Parameters of texture profile analysis and ink print test (IPT) of market *idli* samples

Market	Texture Profile Analysis						IPT
	Hardness (N)	Adhesiveness (N. s)	Springiness (mm)	Cohesiveness	Chewiness (J)	Resilience	NOP
1	$2.617^{j}\pm0.18$	$-.004^{h}\pm0.002$	$.846^{f}\pm0.02$	$0.777^{fgh}\pm0.06$	$1.706^{f}\pm0.07$	$.294^{l}\pm0.016$	$106.667^{f}\pm0.33$
2	$2.954^{ij}\pm0.35$	$-.009^{g}\pm0.003$	$.854^{ef}\pm0.01$	$0.865^{bcd}\pm0.03$	$2.184^{e}\pm0.32$	$.320^{e}\pm0.01$	$96.667^{g}\pm0.5$
3	$5.128^{c}\pm0.99$	$-.022^{d}\pm0.01$	$.873^{c}\pm0.01$	$0.938^{a}\pm0.05$	$4.148^{b}\pm0.50$	$.340^{b}\pm0.01$	$79.333^{h}\pm0.5$
4	$2.466^{k}\pm0.20$	$0^{i}\pm0$	$.887^{a}\pm0.01$	$0.806^{efgh}\pm0.01$	$1.773^{f}\pm0.17$	$.305^{i}\pm0.004$	$145.333^{a}\pm0.5$
5	$4.156^{e}\pm0.19$	$-.016^{f}\pm0.001$	$.812^{j}\pm0.03$	$0.832^{de}\pm0.001$	$2.810^{d}\pm0.24$	$.300^{k}\pm0.005$	$127.667^{d}\pm0.5$
6	$3.872^{e}\pm0.06$	$-.020^{e}\pm0.006$	$.879^{b}\pm0.02$	$0.961^{a}\pm0.04$	$3.267^{c}\pm0.02$	$.373^{a}\pm0.02$	$114^{e}\pm0.5$
7	$8.054^{a}\pm0.34$	$-.059^{a}\pm0.01$	$.855^{e}\pm0.01$	$0.873^{bc}\pm0.01$	$6.016^{a}\pm0.43$	$.321^{d}\pm0.01$	$78.667^{i}\pm0.5$
8	$4.566^{d}\pm0.06$	$-.031^{b}\pm0.01$	$.868^{d}\pm0.02$	$0.830^{def}\pm0.03$	$3.288^{c}\pm0.15$	$.303^{j}\pm0.01$	$136.333^{b}\pm0.5$
9	$3.626^{f}\pm0.20$	$-.016^{f}\pm0.003$	$.884^{a}\pm0.004$	$0.897^{b}\pm0.07$	$2.869^{d}\pm0.07$	$.324^{c}\pm0.03$	$130.333^{c}\pm0.5$
10	$6.564^{b}\pm0.15$	$-.028^{c}\pm0.001$	$.787^{j}\pm0.01$	$0.829^{defg}\pm0.03$	$4.277^{b}\pm0.11$	$.319^{f}\pm0.01$	$127.667^{d}\pm0.5$

IPT- ink print test; NOP- number of pores.

Mean ± SE, Mean values with different superscripts are significantly different with each other ($p<0.05$) within a same column.

CD (Hardness)=0.466 CD (Adhesiveness)=0.001 CD (Springiness)=0.025
CD (Cohesiveness)=0.046 CD (Chewiness)=0.327 CD (Resilience)= 0.001
CD (IPT)= 0.557

In the past, Lavanya and Pinky (2019) used the 9-point hedonic scale to analyze the sensory parameters of rice-urad dal based *idli*, such as color, appearance, aroma, texture, taste, and overall acceptability. This study noted the scores of all sensory parameters, namely color (8.10 ± 0.18), appearance (8.10 ± 0.18), aroma (7.60 ± 0.22), texture (7.10 ± 0.31), flavor (7.50 ± 0.22) and

general acceptability. (7.68 ± 0.16) For the control *idli* (*idli* based on parmal variety rice), these scores are higher than other *idli*-based rice varieties (HB2, HKR48 and HRK128). The scores of the market samples in present study also attained the scores close to those mentioned in the previous research.

Table 4.4. Ranges of values for sensory scores, parameters of texture profile analysis and number of pores for different market sample

Sl. No	Parameters	Ranges (minimum to maximum)
Sensory		
1	Color	2.50 to 7.20
2	Dryness of interior	4.23 to 6.50
3	Porosity	4.60 to 7.00
4	Firmness	2.83 to 7.20
5	Springiness	3.47 to 7.03
6	Fermented aroma	1.00 to 8.37
7	Acidic taste	1.37 to 7.90
8	Overall acceptability	5.07 to 7.77
Parameters for Textural profile analysis		
9	Hardness (N)	~2.5 to ~8
10	Adhesiveness (N.s)	~ -0.5 to 0
11	Springiness (mm)	0.787 to 0.884
12	Cohesiveness	0.777 to 0.961
13	Chewiness (J)	1.706 to 6.016
14	Resilience	0.294 to 0.73
Ink print test for number of pores		
15	Number of pores	78 to 145

4.2 Proximate analysis of Rice, Urad Dhal, Sorghum and *Moringa* Pod Powder

The content of protein, fat, ash, moisture and carbohydrates (by difference method) of RE, UD, SR and MPP was determined, and the mean and standard error (SE) are given in Table 4.5. Zubair *et al.*, (2012) analyzed the proximate composition of different varieties of rice and reported the values for moisture (~ 7 to 9 g), carbohydrates (~ 78 g), proteins (~ 7.5 to 9), fats (~ 1.9 to 2.7) and ash (~ 1.4 to 2g) per 100g. Girish *et al.*, (2012) found moisture (~ 11%), carbohydrates (~ 58%), protein (~ 24%), fat (~ 1.8%), and ash (~ 3.24%) in black gram cotyledons. Udachan *et al.*, (2012) observed the proximate composition of different varieties of sorghum grown in India, namely moisture (~ 8 to 10%), carbohydrate (~ 70 to 76%), protein (~ 8.9 to 11%), fat (~ 2.3 to

2.8 %) and ash content (~ 0.9 to 1.8%). The values obtained in present study (Table 4.5) are in accordance with those reported earlier.

Table 4.5. Proximate constituents of RE, UD, SR and MPP

Chemical constituents	RE	UD	SR	MPP
Moisture content (%)	$11.2^{a} \pm 0.10$	$8.9^{c} \pm 0.13$	$10.3^{b} \pm 0.10$	$4.6^{d} \pm 0.16$
Carbohydrate (%) (Difference method)	$78^{a} \pm 0.43$	$61.1^{b} \pm 0.31$	$77.5^{a} \pm 0.38$	$53.93^{c} \pm 0.92$
Protein (%)	$7.6^{d} \pm 0.38$	$22.3^{a} \pm 0.21$	$8.5^{c} \pm 0.27$	$19.6^{b} \pm 0.39$
Fat (%)	$2.7^{c} \pm 0.13$	$4.5^{b} \pm 0.15$	$2.2^{c} \pm 0.00$	$15.5^{a} \pm 0.55$
Ash (%)	$0.52^{d} \pm 0.00$	$3.24^{b} \pm 0.04$	$1.46^{c} \pm 0.01$	$6.37^{a} \pm 0.07$

Mean ± SE (n=3), Mean values with different superscripts are significantly different with each other ($p<0.05$) within a same row.

4.3 Drying curve for different grains

Based on the optimized process steps for preparation of instant *idli* mix, the grains were soaked for natural fermentation for fixed period of time. The grains were dried in a cabinet tray and drying curve was obtained. Instant mixtures or ready to cook mixes are the dry mixtures available in the market. Typically, instant dry blends contain starch as the main ingredient with low moisture content (Luallen, 2018). The low moisture content extends the shelf life from 6 months to a year at room temperature, providing comprehensive enzymatic browning protection, oxidation and flavor stability (Haleem and Omran, 2014).

4.3.1 Drying curve for rice

Drying curve for rice was plotted between moisture content vs time; moisture loss vs time and drying rate vs free moisture as given in Figures 4.1 (a) and (b), respectively. It can be observed from Fig 4.1(a) that decrease in moisture content occurred as time increased from 0 min to 210 min. The moisture loss with respect to time increased initially between 0 min to 90 min due to removal of free moisture content. The moisture loss reduced from 90 to 120 minutes. This could be due to removal of bound water from internal structure of rice grains.

It can be seen from Fig. 4.1(b) that the unbound or free moisture was removed from rice during the time period of 0 min to 60 min and this zone A to B could be noted as preliminary warming up period where the hot air resulted in latent heat of vaporization causing moisture removal from rice. Next, B to C zone was observed as constant rate period where the drying rate was constant i.e., about 0.43 kg of water/ kg of dry matter in hr. Further, rate of drying decreased

(from 0.43 to 0.03 kg water/kg of dry matter in hr) during 90 min to 210 min i.e., C to E region. The C to E zone was divided into 2 falling rate periods. (i) Region C to D could be considered first falling rate period (60 min to 90 min) when product surface remains wet (Francis and Peters, 1980) because the diffusion of water vapor through the boundary film of air occurred from surface to center (inside) of product in the 1st falling rate period, and (ii) D to E region could be considered as second falling period for rice where rice was dried completely (120 min to 210 min). The partial pressure of water in food is below the saturated vapor pressure at second falling rate period (Srikiatden and Roberts. 2006). Finally, Equilibrium Moisture Content (EMC) of rice was attained i.e., 3.4% and which was observed by development of dry surface/ spots on rice kernels.

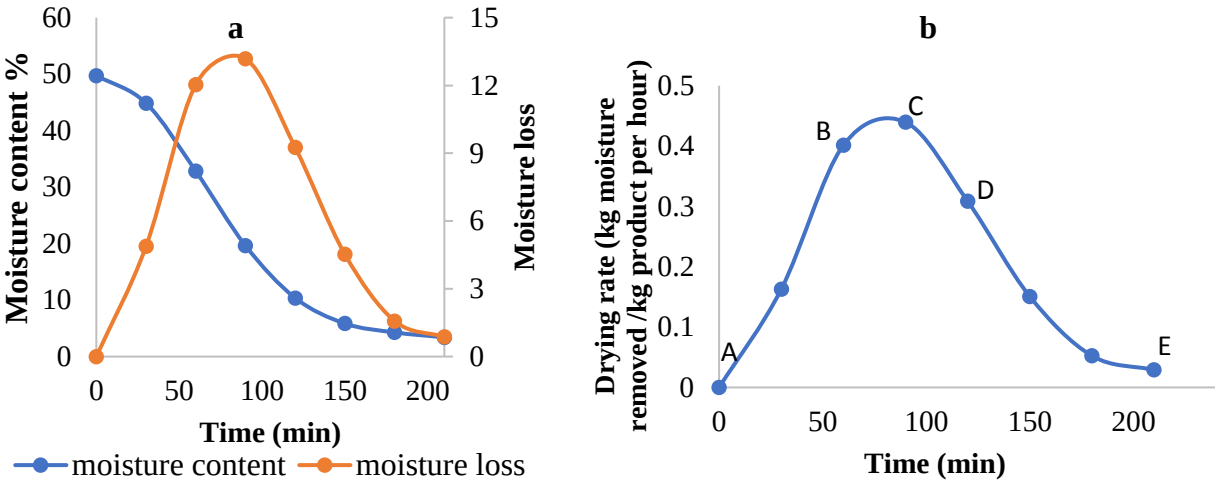


Fig 4.1 Drying curve of rice [(a) Typical drying curve and (b) Drying rate based drying curve]

4.3.2 Drying curve for Urad dhal

The drying curve for urad dhal was plotted between moisture content vs time; moisture loss vs time and drying rate vs free moisture and is shown in Figures 4.2 (a) and (b), respectively. It can be observed from Fig 4.2 (a) that decrease in moisture content occurred as time increased from 0 min to 210 min. However, the rate of moisture loss was higher from 0 to 30 min and 60 to 90 min with respect to time. This could be due to removal of free moisture and loosely bound water during this particular period. The rate of moisture removal decreased subsequently from 90 to 210 mins which could be due to removal of bound water from internal structure of urad dhal.

It can be seen from Fig. 4.2 (b) that the unbound or free moisture was removed from urad dhal during the time period of 0 min to 30 min and this zone A to B and C to D was reported as preliminary warming up period where the hot air resulted in latent heat vaporization for removal of moisture present in fermented urad dhal because of presence of free water and loosely bound water. The same kind of drying curve was obtained in the drying of minced meat (Earle, 1983). Next, B to C zone was observed as constant rate period and where the drying rate was constant i.e., about ~ 0.8 kg of water/ kg of dry matter in hr. Further, rate of drying was decreased (from 1.36 to 0.03 kg water/kg of dry matter in h) during 60 min to 210 min from D to F region. The D

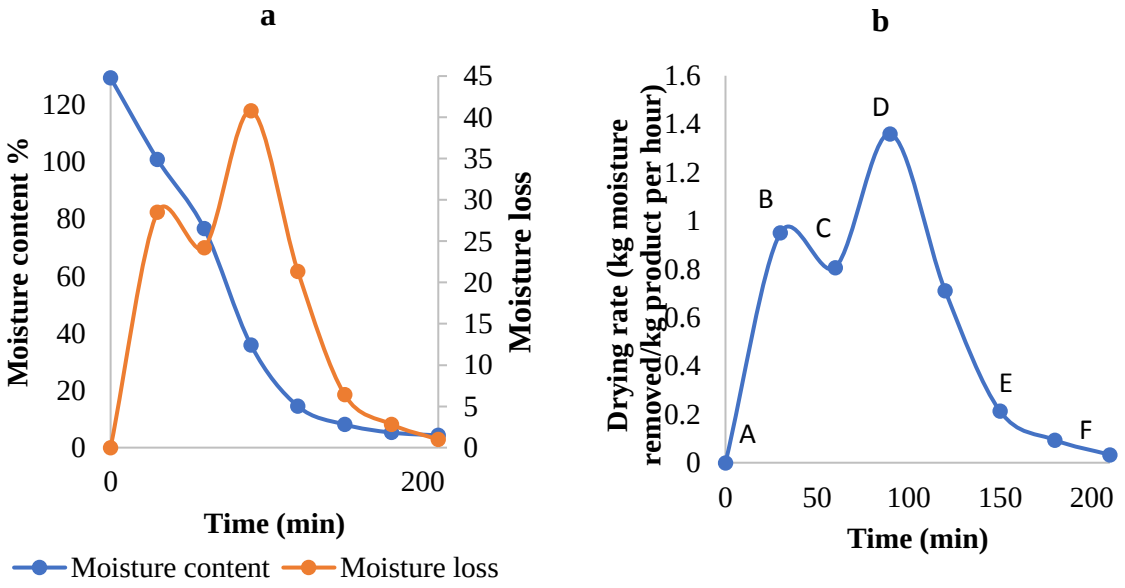


Fig 4.2 Drying curve of Urad dhal [(a) Typical drying curve and (b) Drying rate based drying curve]

to F zone was divided into 2 falling rate periods, namely (i) Region D to E which could be considered first falling rate period (60 min to 120 min) when product surface remained wet (Francis and Peters, 1980) because the diffusion of water vapor through the boundary film of air took place from surface to center (inside) of product in the 1st falling rate period and (ii) E to F region could be considered as second falling period for urad dhal where it dried completely (120 min to 210 min). The partial pressure of water in food is below the saturated vapor pressure at second falling rate period (Srikiatden and Roberts. 2006). Finally, Equilibrium Moisture Content (EMC) of urad dhal was attained i.e., 4.29% and which was observed by developed dry spots/ surface on urad dhal kernels.

4.3.3 Drying curve for sorghum

Drying curve for fermented sorghum was plotted between moisture content vs time; moisture loss vs time and drying rate vs free moisture is given in Figure 4.3(a) and (b), respectively. It can be observed from Fig 4.3(a) that decrease in moisture content of sorghum grain occurred as the time of drying increased from 0 mins to 210 mins. But moisture loss was higher during the initial phase of drying i.e., from 0 to 30 min where free moisture got removed from the sorghum grains. The rate of moisture loss was lower from 30 to 210 minutes, which could be due to removal of bound water from internal structure of grain.

It can be seen from Fig.4.3 (b) that the unbound or free moisture was removed from sorghum during the time period of 0 min to 30 min and this zone A to B noted as preliminary warming up period where the hot air caused the latent heat vaporization on the moisture which presented in sorghum. Next, C is observed as constant rate period for drying and where the drying

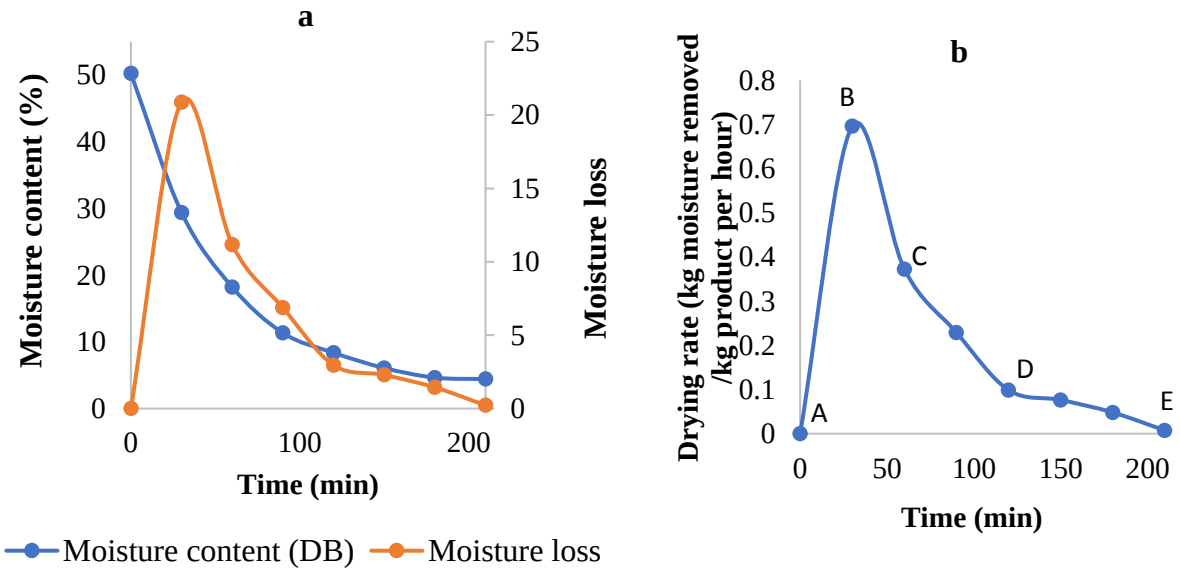


Fig 4.3 Drying curve of Sorghum [(a) Typical drying curve and (b) Drying rate based drying curve]

rate was constant i.e., about 0.3 kg of water/ kg of dry matter in hr. Further, rate of drying was decreased (from 0.22 to 0.007 kg water/kg of dry matter in hr) during 90 min to 210 min between C to E region. The C to E zone was divided into 2 falling rate periods, i.e., (i) Region C to D could be considered first falling rate period (90 min to 150 min) when product surface remained wet (Francis and Peters, 1980) because the diffusion of water vapor through the boundary film of air

occured from surface to center (inside) of product in the 1st falling rate period and (ii) D to E region could be considered as second falling period for sorghum where sorghum was dried completely (150 min to 210 min). Finally, Equilibrium Moisture Content (EMC) of sorghum was attained i.e., 4.4% and which was observed by development of dry spots/ surface on sorghum kernels.

4.4 Optimization of the method for preparation of product

A number of approaches were adopted for finalization of procedure for preparation of instant *idli* mix. The approaches for optimization of pre-treatments included (i) pre-gelatinization by (a) dry roasting and (b) wet pre-gelatinization (hydrothermal treatment); (ii) addition of culture for instant fermentation and (iii) natural fermentation of grains. The proposition of major ingredients used for optimization of processing steps are given in Table 4.6.

4.4.1 Preliminary treatments for pre-gelatinization of grains

Pregelatinized starch is a very significant functional ingredient in instant or ready-to-eat food. Pre-gelatinization is a process of modifying starch using various technologies (Guaras *et al.*, 2017), which can improve the functional properties of starch i.e., high hygroscopicity (Goyat *et al.*, 2020) and affect the physical and chemical properties of starch, such as increasing swelling capacity, solubility, gelatinization temperature and starch paste stability, which can be used to develop new food products (Jacobasch *et al.*, 2006 and Wijanarka *et al.*, 2017). Pre-gelatinization based instant mixes are easily reconstituted with water due to high hygroscopicity of ingredients.

4.4.1. 1 Dry roasting

Sand roasting enhances the digestibility of protein and starch by break down of its macromolecules and can form resistant starch making it suitable for children and diabetic people, respectively (Kora, 2019). Sneha and Hari Priya (2018) developed an instant dosa mix by dry roasting of amaranth and bengal gram flour. They found that the roasted-based dosa mix had high crude fiber content and lower protein than the control dosa mix. In present study, the grains were ground and dry roasted in ghee and formulated into an *idli* mixture for optimization of processing steps. The *idli* produced by this method was hard and the panelists marked it as unacceptable in preliminary sensory analysis.

4.4.1.2 Wet pre-gelatinization (hydrothermal treatment)

Wet pre-gelatinization is one of the easiest methods for producing pre-gelatinized starch. Hydrothermal treatment is the method of wet based pre-gelatinization of grains. For example,

paddy is parboiled to produce parboiled rice, which increases the head rice recovery and rice kernel containing majorly starch, that is pre-gelatinized. Starch swells in water and can replace the gluten

Table 4.6 Propositions of ingredients used for optimization of processing steps

Ingredients	Parts
Rice	1.5 parts/21.43%
Urad dhal	1 part/14.29%
Sorghum	4.5 Parts/64.29%

(Rheological property) in dough (Chilo *et al.*, 2009). This could be done through wet based pre-gelatinization. Therefore, hydrothermal process was used to optimize processing steps as previous approached did not yield an acceptable *idli* sensorially. Grains like urad dhal and sorghum were soaked for 4 hours and thermal treatment was done by heating grains in water as per previous studies i.e., UD and SR parboiled in boiling water for 8 minutes (Gala'n and Drago, 2018) and 10 minutes, respectively. But pre-gelatinization of grains in water showed more stickiness on FMID during preliminary sensory evaluation. Rohaya *et al.*, (2013) noted that heating of starch granules in water affected gelatinization process as it leads to swelling of starch granules and producing viscous paste. Hence, the grains/ seeds were pre-gelatinized by soaking followed by treating with steam (indirectly). The indirect hydrothermal treatment by steam produced functional multi-grain *idli* (FMID) which did not show any stickiness. However, the panelists reported that prepared *idli* possessed little hardness. Like-wise, ghee-roasting based FMID resulted in harder FMID than FMID produced after the hydrothermal process as per the preliminary trials of sensory evaluation.

4.4.2 Optimization of soaking time for hydrothermal pre-gelatinization method

It can be inferred from the discussions held in the preceding section that hydrothermal treatment employing steam yielded a superior product sensorially. Therefore, the optimization of soaking time for different grains was carried out based on the percent increase in the weight of grains after every half an hour. Table 4.7 showed that weight of urad dhal increased significantly ($p<0.05$) up to 2 hours of soaking but from 2 to 4.5 hours the increase in weight was non-significant ($p>0.05$). However, the weight of sorghum and fenugreek increased significantly ($p<0.05$) up to 4.5 hours of soaking. Therefore, based on statistical analysis 2 hours was selected as soaking time for UD and 4.5 hours was selected for sorghum and fenugreek. Thus, hydrothermal treatment involving steam could be selected after the predefined soaking period. However, other approaches were also tried as indicated in subsequent sections before finalization of the processing steps.

Table 4.7 Effect of soaking time on grains weight

Soaking time	Increased weight (g)		
	Urad dhal	Sorghum	Fenugreek
Initial weight	10±0.01	10±0.05	10±0.12
0 hour	0.00^{g} ±0.00	0.00^{i} ±0.00	0.00^{i} ±0.00
0.5 hour	14.35^{f} ±0.16	2.23^{h} ±0.04	1.39^{h} ±0.00
1.5 hour	16.15^{de} ±0.07	2.87^{g} ±0.00	4.45^{g} ±0.11
2 hours	16.20^{de} ±0.01	3.50^{f} ±0.00	6.68^{f} ±0.01
2.5 hours	16.22^{d} ±0.02	3.98^{e} ±0.01	7.43^{e} ±0.17
3 hours	16.52^{bc} ±0.02	4.23^{d} ±0.15	7.61^{d} ±0.08
3.5 hours	16.53^{bc} ±0.02	4.76^{c} ±0.04	7.87^{c} ±0.04
4 hours	16.56^{ab} ±0.01	5.03^{b} ±0.10	8.47^{b} ±0.02
4.5 hours	16.62^{a} ±0.02	5.30^{a} ±0.04	8.60^{a} ±0.06

Mean ± SE, Mean values with different superscripts are significantly different with each other ($p<0.05$) within a same column.

CD (UD) =0.08 CD (SR)=0.09 CD (Fenugreek)= 0.11

4.4.3 Addition of culture for instant fermentation

In present study, the method was used as one of the approaches for preparation of instant *idli* mix as instant fermentation could yield a superior flavour besides delivering some bio-active components as a secondary metabolite of fermentation. The cultures selected for obtaining instant fermentation were *Propioniibacterium freudenreichii ssp. shermanii* and *Lactobacillus ruteri*. The results indicated that *Propioniibacterium freudenreichii ssp. shermanii* did not grow in media (broth as well as skim milk) aerobically, on the other hand *Lactobacillus ruteri* produced CO_2 in batter only after 1.5 hours of incubation at 38°C which continued till 5.5 hours. But, this CO_2 could neither increase volume of batter nor resulted in fluffy *idli*. This could be due to the reason that CO_2 could not be retained by the batter (Plate 4.1) which resulted in non-fluffy *idli*. Therefore, this approach for preparation of *idli* mix was withdrawn from present research.

Fermented and dehydrated ready mixes for dosa and *idli* batter was developed by CFTRI (Central Food Technological Research Institute), Mysuru. In this product development, rice (raw and parboiled) and black gram dhal were soaked, ground, mixed, fermented, blended, dried, blended with leavening agents, re-ground and packed. Chelliah *et al.*, (2017) studied that the acceleration of fermentation of batter by addition of finger millet powder and peal millet powder

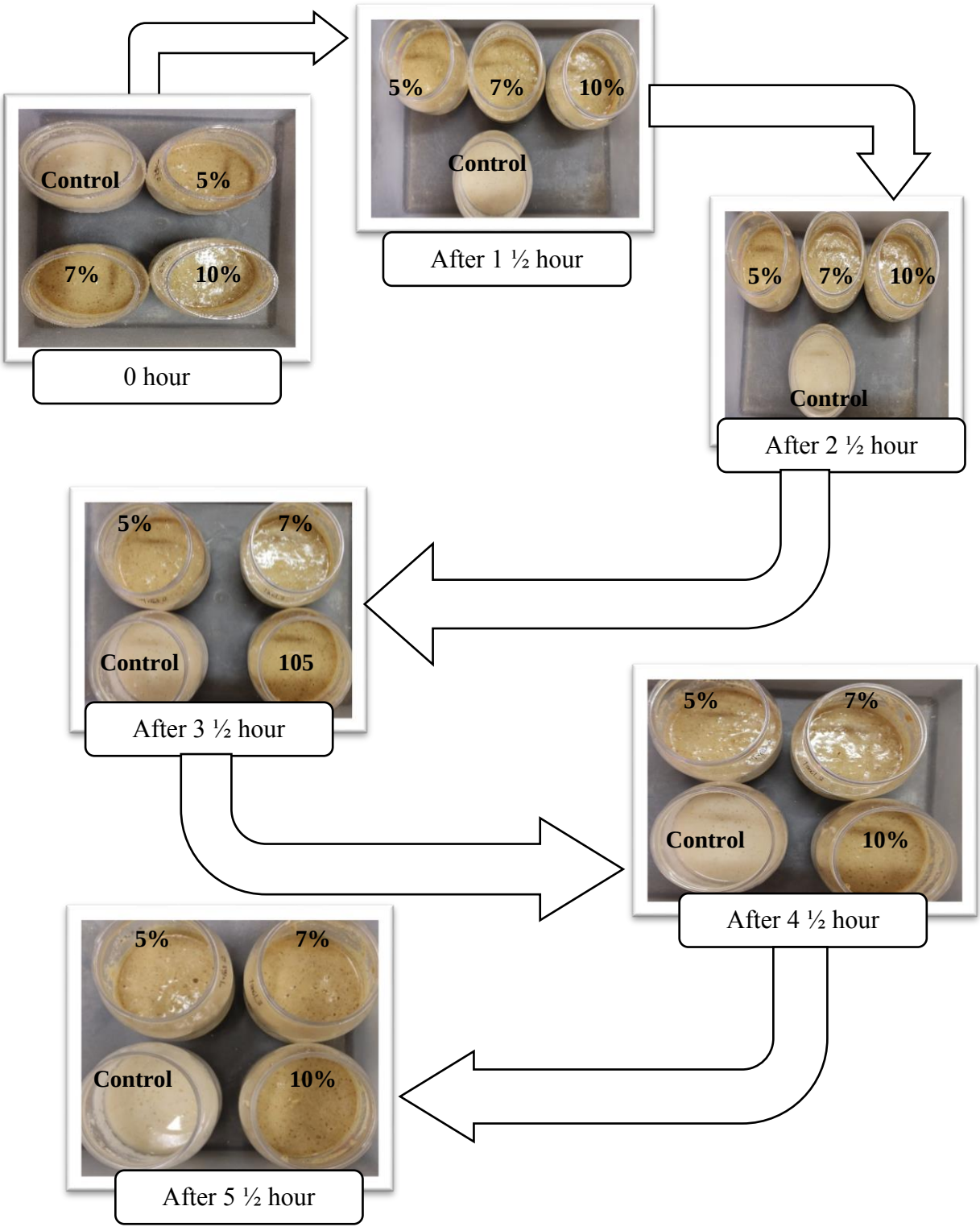


Plate 4.1 *Idli* batter fermentation after addition of culture up to 10%

into unfermented batter, thereby decreasing fermentation time from 12h to 6h and 8h, respectively. Less fermentation time plays a crucial role on large scale level. Hence, biological and chemical leavening agents were utilized for producing instant mixes. i.e., yeast (*Saccharomyces cerevisiae*) and $NaHCO_3$ or eno with organic edible acids (Bishnoi *et al.,* 2015).

Ohariya *et al.,* (2017) prepared instant kodo-soy *idli* mix with yeast and $NaHCO_3$. In the present work, yeast took almost 1 h to ferment the batter and CO_2 production, while $NaHCO_3$ produced CO_2 and leavened the *idli* batter almost instantly with water and heat. Since, the product possessing desired sensorial properties was not obtained through any of the two approaches as indicated above, therefore the third approach of natural fermentation was adopted in anticipation that this could yield a product superior in flavour and texture.

4.4.4 Optimization of fermentation time of grains

The different grains i.e., rice, sorghum and urad dhal were fermented at *°C for different time periods. The fermented grains were used for preparation of *idli* which was subsequently analyzed for sensory evaluation. Based on the sensory analysis (i.e., taste and texture), ** hours of fermentation at *°C was selected. It was further observed that *idli* obtained through this approach was superior in comparison to *idli* obtained after adopting the other two approaches. Therefore, after several processing preliminary trials as indicated in Chapter 3, the final optimized processing steps were finalized. Fermentation of grains for a period of ** hours prior to formulation of *idli* mix was used in the subsequent part of the research for optimization of level of ingredients.

The pictures of *idli* obtained from various approaches and trials is given from Plate 4.2 to Plate 4.4.

4.4.5. Optimization of level of adjuncts to improve taste and texture of Functional Multi-grain *Idli* (FMID)

The levels and/ or type of various ingredients, namely salt, leavening agent and acid, hydrocolloids, etc was fixed based on the preliminary trials. Adjuncts are very important to formulate an optimum *idli* to simulate naturally fermented *idli*. It has been noted by Chelliah *et al.,* (2017) that the instant *idli* pre-mixes do not provide the desired textural characteristics and fermented taste. Hence, there is need of addition of adjuncts to overcome the undesirable textural and taste issues in instant *idli*.

Plate 4.2 (a) Steamed *idli* from dry roasted mix

Plate 4.2 (b) Interior of steamed *idli* from dry roasting approach

Plate 4.3 (a) Steamed *Idli* from wet pre-gelatinized mix

Plate 4.3 (b) Interiors of steamed *idli* from wet pre-gelatinized mix

Plate 4.4 (a) *Idli* from selected approach

Plate 4.4 (b) Interior section of *Idli* from selected approach

The different levels of salts (range and final selection is not revealed due to IPR issues) were used for preparation of *idli* and based on the preliminary studies, one level was selected for further studies. Ohariya *et al.*, (2017) optimized 2% of salt from the levels ranging between 0.8% and 3% in kodo-soy based instant *idli* mix, based on acceptable flavour. A range of leavening agents like $NaHCO_3$, $CaCO_3$, K_2CO_3, NH_4CO_3, etc at various levels (range and final selection is not revealed due to IPR issues) level with different leavening acids i.e. citric acid, malic acid and tartaric acid or Glucono-delta-lactone (GDL) solely as well as in combination in different level (range and final selection is not revealed due to IPR issues) were used as an ingredient with the aim of obtaining original acidic taste of naturally fermented *idli*. The selected combination at a fixed level was used for preparation of optimized product. Gum acacia or gum Arabic, guar gum, I- carrageenan, xanthan gum, carboxymethyl cellulose (CMC) were used singly in preliminary

trials. One of the hydrocolloids was selected as it yielded fluffy texture of FMID. Hydrocolloids are used to improve the texture of *idli* and maintains the stability of batter. Ananthanarayan and Singhal (2004) found that addition 0.1% guar gum improved batter stability at a level of 0.1% and 0.2% (i.e., whey separation). Hydrocolloids also act as surface active agents in gluten free breads and act as texture improver (Yano, 2019). The adjuncts were used at a selected level for optimization of major ingredients for preparation of *idli* mix.

4.5. Optimization of *idli* mix based on Texture profile and Sensory parameters

The optimized levels of adjuncts were used for preparation of instant *idli* mix, while the other ingredients were optimized based on the 2^5 factorial experiment. Thus, five different ingredients were taken at two different levels, making a total of 32 combinations which were analyzed for textural attributes and descriptive sensorial profiling with the aim to optimize one combination for preparation of final product.

4.5.1 Optimization based on textural attributes

Texture refers to those qualities of food that can be felt with the fingers, tongue, palate or teeth and it is also an index of food quality. The effect of different levels of RE, UD, SR, MPP and CR on the textural properties like hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience were studies and are discussed in the subsequent section/ subsection. The sample of FMID was evaluated for its textural attributes using two bite compression tests by Texture Analyzer TAXT2i (Stable Micro System, Godalming, Survey, UK) fitted with 25 kg load cell. Besides, texture profile analysis, softness of *idli* was analyzed by determination of number pores/ square meter which was analyzed through ink print test.

4.5.1.1 Hardness

Hardness is represented by the first peak in the graph or maximum force required at first compression. It is expressed in Newtons. Shobha and Joshi (2019) advocated that the ratio of rice to urad dhal decides the hardness of *idli* and they found hardness of 20.11 N in *idli* prepared from rice and urad dhal having ratio of 3 to 1. As per Table 4.8, the maximum force required for first compression was ~8N obtained from RE, UD, SR, MPP and CR used at levels of Q, R, T, W parts and X g respectively (T10), while the minimum hardness of ~2.3N was obtained from the combinations of RE, UD, SR, MPP and CR at levels Q, R, T, V parts and Y g, respectively (T25). It is clearly explained in Annexure 1 that parts of RE, UD, SR, MPP and CR, showed

Table 4.8: Effect of different levels of RE, UD, SR, MPP and CR on textural properties of FMID

Treatments	RE	UD	SR	MPP	CR(g)	Texture Profile Analysis						Ink Print Test
						Hardness (N)	Adhesiveness (N.s)	Springiness(mm)	Cohesiveness	Chewiness(J)	Resilience	Number of pores
T1	P	R	T	V	X	6.48±0.40	- 0.004± 0.001	0.68±0.02	0.92±0.25	4.19±1.34	0.83±0.32	91.67±0.88
T2	P	R	T	W	X	6.73±0.77	-0.018±0.001	0.59±0.02	0.63±0.01	2.55±0.36	1.51±0.05	93±15
T3	P	S	T	V	X	4.54±0.20	-0.003±0.001	0.77±0.01	0.60±0.03	2.11±0.11	0.21±0.01	97.67±0.33
T4	P	S	T	W	X	4.62±0.07	-0.006±0.001	0.75±0.01	0.71±0.05	2.50±0.19	0.24±0.02	81.67±0.88
T5	P	R	U	V	X	3.81±0.06	-0.003±0.001	0.74±0.03	0.45±0.12	1.33±0.40	0.74±0.55	95.33±1.20
T6	P	R	U	W	X	5.44±0.24	-0.013±0.001	0.72±0.02	0.64±0.05	2.54±0.12	0.22±0.02	80±1.15
T7	P	S	U	V	X	4.39±0.01	-0.001±0.001	0.68±0.01	0.20±0.01	0.61±0.02	2.20±0.25	104.67±1.76
T8	P	S	U	W	X	5.99±0.23	-0.002±0.001	0.73±0.02	0.62±0.03	2.75±0.13	0.21±0.01	111.67±1.45
T9	Q	R	T	V	X	5.31±0.25	-0.012±0.001	0.73±0.01	0.70±0.02	2.76±0.17	0.25±0.00	141±1.15
T10	Q	R	T	W	X	8.09±0.18	-0.018±0.010	0.75±0.01	0.62±0.02	3.80±0.11	0.21±0.01	126.67±1.20
T11	Q	S	T	V	X	5.76±0.02	-0.009±0.001	0.77±0.02	0.97±0.51	4.47±2.40	0.92±0.67	106.33±1.20
T12	Q	S	T	W	X	3.50±0.11	-0.002±0.001	0.80±0.01	0.73±0.05	2.07±0.10	0.27±0.02	66.67±1.86
T13	Q	R	U	V	X	4.82±0.25	-0.008±0.001	0.76±0.00	0.56±0.02	2.10±0.18	0.20±0.01	71±1.15
T14	Q	R	U	W	X	5.71±0.24	-0.002±0.001	0.77±0.02	0.69±0.03	3.06±0.27	0.25±0.01	98.67±1.45
T15	Q	S	U	V	X	3.39±0.15	-0.003±0.001	0.81±0.01	0.59±0.03	1.64±0.02	0.21±0.01	102.67±1.20
T16	Q	S	U	W	X	3.29±0.09	0±0	0.74±0.04	0.45±0.14	1.16±0.40	0.72±0.51	87±1.15
T17	P	R	T	V	Y	4.76±0.27	-0.056±0.02	0.68±0.02	0.38±0.04	1.23±0.16	0.15±0.02	72.33±1.45
T18	P	R	T	W	Y	3.65±0.11	0±0.00	0.72±0.01	0.34±0.02	0.92±0.04	0.17±0.02	103±1.15
T19	P	S	T	V	Y	3.9±0.22	-0.003±0.001	0.78±0.01	0.38±0.04	1.19±0.15	0.20±0.03	105.67±1.76
T20	P	S	T	W	Y	2.52±0.19	0±0	0.62±0.03	0.37±0.02	0.59±0.08	0.19±0.01	91.33±0.88
T21	P	R	U	V	Y	3.08±0.45	-0.006±0.010	0.56±0.01	0.37±0.02	0.66±0.13	0.18±0.01	94.67±0.33
T22	P	R	U	W	Y	5.07±0.53	-0.006±0.001	0.60±0.05	0.34±0.04	1.06±0.16	0.16±0.03	78.67±0.67
T23	P	S	U	V	Y	4.32±0.03	-0.041±0.020	0.59±0.02	0.35±0.03	0.92±0.11	0.17±0.01	46±0.58
T24	P	S	U	W	Y	5.11±0.46	-0.011±0.001	0.56±0.02	0.34±0.03	1.00±0.10	0.17±0.02	86.67±0.88
T25	Q	R	T	V	Y	2.33±0.04	0±0.00	0.71±0.00	0.34±0.01	0.57±.0.01	0.18±0.01	**67.33±0.67**

Treatments	RE	UD	SR	MPP	CR(g)	Hardness (N)	Adhesiveness (N.s)	Springiness(mm)	Cohesiveness	Chewiness(J)	Resilience	Number of pores
T26	Q	R	T	W	Y	3.07±0.12	-0.018±0.010	0.66±0.01	0.43±0.07	0.88±0.14	0.19±0.03	97.33±0.67
T27	Q	S	T	V	Y	**3.31±0.13**	**-0.016±0.010**	**0.66±0.01**	**0.38±0.02**	**0.85±0.07**	**0.20±0.01**	**111±0.58**
T28	Q	S	T	W	Y	3.53±0.12	-0.009±0.00	0.63±0.01	0.34±0.02	0.77±0.04	0.17±0.01	103.33±0.33
T29	Q	R	U	V	Y	3.50±0.23	-0.030±0.02	0.69±0.01	0.36±0.01	0.87±0.07	0.18±0.000	111.67±0.88
T30	Q	R	U	W	Y	3.19±0.24	-0.008±0.01	0.68±0.02	0.34±0.03	0.74±0.06	0.17±0.02	92±0.58
T31	Q	S	U	V	Y	3.19±0.02	-0.002±0.001	0.74±0.01	0.36±0.02	0.87±0.06	0.19±0.01	83.67±0.67
T32	Q	S	U	W	Y	3.32±0.27	-0.006±0.001	0.68±0.02	0.30±0.01	0.69±0.01	0.15±0.00	92.67±1.45

FMID- Functional Multi grain *idli*

For hardness CD (RE*UD*SR*MPP*CR) = 0.748
For adhesiveness CD (RE*UD*SR*MPP*CR) = 0.001
For springiness CD (RE*UD*SR*MPP*CR) = 0.163
For cohesiveness CD (RE*UD*SR*MPP*CR) =0.309
For chewiness CD (RE*UD*SR*MPP*CR) = 1.450
For resilience CD (RE*UD*SR*MPP*CR) = 0.541
For no. of pores CD (RE*UD*SR*MPP*CR) = 3.131

Table 4.9. Effect of different levels of RE, UD, SR, CR and MPP individually on textural parameters of FMID

Ingredient	Parts	Texture profile Analysis						IPT
		Hardness	Adhesiveness	Springiness	Cohesiveness	Chewiness	Resilience	Number of pores
RE	P	4.654^{a}	-0.009^{a}	0.680^{a}	0.482^{a}	1.638^{a}	0.477^{a}	89.625^{b}
	Q	4.013^{b}	-0.008^{a}	0.733^{a}	0.515^{a}	1.699^{a}	0.284^{b}	95.188^{a}
UD	R	4.694^{p}	-0.010^{p}	0.696^{p}	0.511^{p}	1.833^{p}	0.355^{p}	94.646^{p}
	S	3.973^{q}	-0.006^{p}	0.717^{p}	0.486^{p}	1.503^{q}	0.406^{p}	90.167^{q}
SR	T	4.434^{m}	-0.007^{m}	0.716^{m}	0.558^{m}	1.958^{m}	0.373^{m}	95.000^{m}
	U	4.233^{n}	-0.009^{m}	0.697^{m}	0.440^{m}	1.378^{n}	0.388^{m}	89.813^{n}
MPP	V	4.185^{y}	-0.010^{x}	0.714^{x}	0.449^{x}	1.652^{x}	0.443^{x}	93.917^{x}
	W	4.482^{x}	-0.007^{x}	0.698^{x}	0.449^{x}	1.685^{x}	0.318^{x}	90.896^{y}
CR	X	5.122^{r}	-0.006^{r}	0.742^{r}	0.635^{r}	2.482^{r}	0.580^{r}	97.229^{r}
	Y	3.545^{s}	-0.010^{r}	0.671^{r}	0.363^{s}	0.855^{s}	0.181^{s}	87.583^{s}

FMID: Functional Multi-grain *Idli*

significantly effect ($p<0.05$) on hardness of FMID individually and in almost all combinations expect, RE and MPP; RE, SR and CR; RE, MPP and CR; UD, SR and CR; SR, MPP and CR; RE, UD, SR and CR; RE, SR, MPP and CR; UD, SR, MPP and CR; and RE, UD, SR, MPP and CR.

It can be observed from Table 4.9 that as the level of RE, UD, SR and CR increased, the hardness of samples decreased (i.e., from 4.65N to 4.013N, 4.694 to 3.973N, 4.434 to 4.233 and 5.1233 to 3.544, respectively), while as the level of MPP increased, hardness also increased (i.e. from 4.18 to 4.48N). This could be due to high amount of fiber in MPP. Table 4.10 showed that the interaction of highest level of RE (Q) with UD (S), SR (U) and CR (Y g) decreased the hardness of FMID. But in contrast, interaction of RE (P) with other ingredient showed highest hardness on FMID at lowest level of these ingredients.

It can be noted from Table 4.11 that least hardness was obtained with interaction of S parts of UD with T parts of SR, W parts of MPP and Y g of CR, while maximum hardness was obtained with R parts of UD with T parts of SR, W parts of MPP and X g of CR. It was interesting to note

Table 4.10 Effect of interaction of RE with UD, SR and CR on hardness of FMID

Level of RE	Level of UD		Level of SR		Level of CR		Overall Mean
	R	S	T	U	X	Y	
P	4.881	4.427	4.651	4.657	5.254	4.055	4.654^{a}
Q	4.507	3.518	4.217	3.808	4.991	3.034	4.013^{b}
Overall Mean	4.694^{x}	3.973^{y}	4.434^{x}	4.233^{y}	5.123^{x}	3.544^{y}	

FMID: Functional Multi-grain *Idli*

CD (RE) =0.187 CD (UD) =0.187 CD (SR) =0.187

CD (CR) =0.187 CD (RE*UD) =0.265 CD (RE*SR) =0.265

CD (RE*CR) =0.265

Table 4.11: Effect of interaction of UD with SR, MPP and CR on hardness of FMID

Parts of UD	Parts of SR		Parts of MPP		Parts of CR		Overall Mean
	T	U	V	W	X	Y	
R	5.056	4.333	4.265	5.213	5.803	3.585	4.694^{a}
S	3.813	4.132	4.105	3.840	4.441	3.504	3.972^{b}
Overall Mean	4.434^{x}	4.233^{y}	4.185^{x}	4.526^{y}	5.122^{x}	3.544^{y}	

FMID: Functional Multi-grain *Idli*

CD (UD) =0.187 CD (SR) =0.187 CD (MPP) =0.187

CD (CR) =0.187 CD (UD*SR) =0.265 CD (UD*MPP) =0.265

CD (UD*CR) =0.265

that UD, SR and CR at higher levels showed least hardness except MPP. MPP at W parts resulted in FMID having highest hardness (Table 4.11 and 4.12). It can be noted from Table 4.12 that highest hardness (5.427 N) was obtained at W parts of MPP and X g of CR, while lowest hardness

(3.53 N) was obtained with the corresponding values of these ingredients of W parts of MPP and Y g of CR.

Table 4.12: Effect of interaction of MPP and CR on hardness of FMID

Parts of MPP	Parts of CR		Overall Mean
	X	**Y**	
V	4.817	3.552	4.184^{b}
W	5.427	3.537	4.482^{a}
Total Mean	5.122^{x}	3.545^{y}	

FMID: Functional Multi-grain *Idli*
CD (MPP) =0.187 CD (CR) =0.187 CD (MPP*CR) =0.265

According to the Tables 4.8, 4.9, 4.10, 4.11 and 4.12, it can be inferred that Q parts RE, S parts UD, T parts SR, W parts MPP and Y g CR could be selected for obtaining optimum hardness value of FMID and this combination of ingredients showed optimum level of hardness i.e., 3.53±0.12 N (Table 4.8). However, if this combination is compared with the one containing U parts of SR while keeping the level of rest of the ingredients as same, the hardness of FMID was obtained to be 3.32±0.27 N (Table 4.8), which also fell within the range established from market samples. Therefore, RE, UD, SR could be selected at Q, S, U parts level, while W parts MPP and Y g CR could be selected considering hardness of *idli*. Maheswari and Shetty (2013) revealed that increasing level of *Moringa oleifera* leaves from 5% to 20% in *idli* batter showed decreased level of hardness on *idli*. The results obtained in present study are in accordance with the reported literature.

4.5.1.2 Adhesiveness

Adhesiveness is represented by the negative peak in the graph or negative force area while first bite and work needed to overcome the attractive forces between food and palate of mouth. It is expressed in N. s. Adhesiveness is directly related to stickiness of product (Durgadevi and Shetty, 2012). It is related to hydration of starch granules (Zhou *et al.*, 2007) and amylose starch in rice absorbs moisture while cooking leads to higher adhesiveness and decreases the hardness (Ghasemi *et al.*, 2009). As per Table 4.8, the maximum adhesiveness (in absolute terms) was ~-0.056 N.s (T17) and obtained from RE, UD, SR, MPP and CR used at levels of P, R, T, V parts and Y g respectively, while the minimum adhesiveness of ~0 N.s was obtained from the combinations of RE, UD, SR, MPP and CR at levels P, R, T, W parts and Y g (T18); P, S, T, W parts and Y g (T20); Q, R, T, V parts and Y g (T25) and Q, S, U, W parts and Y g (T32), respectively. It is clearly explained in Annexure 1 that parts of RE, UD, SR, MPP and CR, showed non-significant effect ($p>0.05$) on adhesiveness of FMID individually and in almost all

combinations expect SR and CR; MPP and CR; RE, UD and SR; RE, UD, SR and MPP; and RE, UD, SR and CR.

It can be observed from Table 4.9 that as the level of RE, UD, and MPP increased, the adhesiveness of samples decreased (i.e., from -0.009 to -0.008 N. s, from -0.010 to -0.006 N.s and from -0.010 to -0.007 N.s, respectively) non-significantly ($p>0.05$), while as the level of SR and CR increased, adhesiveness also increased (i.e., from -0.007 to -0.009 N.s and from -0.006 to -0.010 N.s, respectively) non-significantly ($p>0.05$).

Table 4.13: Effect of interaction of combination of CR with SR and MPP on adhesiveness of FMID

Level of CR	**Level of SR**		**Level of MPP**		**Overall Mean**
	T	**U**	**V**	**W**	
X	-0.009	-0.004	-0.005	-0.008	-0.007^{a}
Y	-0.006	-0.014	-0.014	-0.006	-0.01^{b}
Overall Mean	-0.008^{x}	-0.009^{y}	-0.01^{y}	-0.007^{x}	

FMID: Functional Multi-grain *Idli*

CD (CR) =0.002 CD (SR)=0.001 CD (MPP) =0.001

CD (CR*SR) =0.001 CD (CR*MPP) =0.002

It can be noted from Table 4.13 that least adhesiveness was obtained with interaction of X g of CR with U parts of SR and X g of CR with V parts of MPP, while maximum adhesiveness was obtained with Y g of CR with U parts of SR and Y g of CR with V parts of MPP. According to the Tables 4.8, 4.9 and 4.13, it can be inferred that U and V parts of SR and MPP with X g CR, could be selected for obtaining optimum level of adhesiveness value of FMID. Individually, RE and UD could be selected at Q and S parts because of their non-significant ($p<0.05$) effect on adhesiveness which led to low adhesiveness (Table 4.8). This combination showed adhesiveness (-0. 003±0.00 N.s, Table 4.8) which also fell within the range established from the market samples. Therefore, RE, UD, SR could be selected at Q, S, U parts, while V parts of MPP and X g CR could be selected considering adhesiveness of FMID.

4.5.1.3 Springiness

Springiness is expressed as ratio or percentage of products height. It is percentage of detected height during the second compression divided by the original compression distance. Generally, springiness is how well a product physically springs back after it has been deformed during the first compression. The springiness value of *idli* depends on the quantity of urad dhal addition for batter preparation. Maheswari and Shetty (2013) noted higher springiness (0.78) in

5% *Moringa oleifera* leaves added *idli* than control (0.57) and similarly replacing of rice portion with maize also showed low springiness (Shobha and Joshi, 2018).

As per Table 4.8, the maximum springiness was ~0.8mm which was obtained from the combinations of RE, UD, SR, MPP and CR at levels of Q, S, T, W parts and X g (T12); and Q, S, U, V parts and X g (T15) respectively. Minimum springiness (~0.5mm) was obtained from the combinations of RE, UD, SR, MPP and CR with levels of P, R, T, W parts, and X g (T2); and and P, S, U, V parts, X g (T7); P, R, U, V parts, and Y g (T21); P, S, U, W parts, and Y g (T24) respectively. It is clearly explained in Annexure 1 that parts of RE, UD, SR, MPP and CR, showed significantly effect ($p<0.05$) on adhesiveness of FMID individually and in almost all combinations expect, dual combination of RE with UD, MPP and CR; dual combination of MPP with RE, UD, SR and CR; and combinations of RE, MPP and CR; UD, SR and CR; SR, MPP, CR; RE, UD, SR and CR; RE, SR, MPP, and CR; UD, SR, MPP, and CR; and RE, UD, SR, MPP and CR.

It can be observed from Table 4.9 that as the level of RE and UD increased, the springiness of samples increased (i.e., from 0.680mm to 0.733mm and from 0.696mm to 0.717mm, respectively) non-significantly ($p>0.05$), while as the level of SR, MPP and CR increased, springiness also decreased (i.e., from 0.71mm to 0.71mm, from 0.71mm to 0.69mm and from 0.74mm to 0.67mm, respectively) non-significantly ($p>0.05$).

Table 4.14: Effect of interaction of combination of SR with RE and UD on springiness of FMID

Level of SR	**Level of RE**		**Level of UD**		**Overall Mean**
	P	**Q**	**R**	**S**	
T	0.705	0.723	0.695	0.738	0.715^{a}
U	0.645	0.743	0.696	0.697	0.695^{b}
Overall Mean	0.675^{y}	0.733^{x}	0.696^{q}	0.718^{p}	

FMID: Functional Multi-grain *Idli*

CD (SR) =0.013 CD (RE) =0.013 CD (UD) =0.013

CD (SR*RE) =0.018 CD (SR *UD) =0.018

It can be observed from Table 4.14 that interaction of T parts of SR with P parts of RE; and T parts of SR with S parts of UD showed maximum springiness i.e., 0.743mm and 0.738mm, respectively; while U parts of SR with P parts of RE; and T parts of SR with Q parts of RE showed minimum springiness. Springiness increased significantly ($p<0.05$) with increasing level of RE and UD

individually and this could be due to the UD which contributed to gas holding capacity of batter during mixing and steaming of FMID.

Table 4.15: Effect of interaction of combination of CR with UD and SR on springiness of FMID

Level of CR	Level of UD		Level of SR		Overall Mean
	R	S	T	U	
X	0.724	0.761	0.735	0.750	0.743^{a}
Y	0.668	0.674	0.698	0.643	0.670^{b}
Overall Mean	0.696^{y}	0.718^{x}	0.717^{p}	0.697^{q}	

FMID: Functional Multi-grain *Idli*

CD (CR) =0.013 CD (UD) =0.013 CD (SR) =0.013

CD (CR * UD) =0.018 CD (CR * SR) =0.018

But decrease in springiness significantly ($p<0.05$) occurred with increased level of SR individually. This could be due to indirectly increasing level of SR while decreasing the level of UD in FMID.

Table 4.15 showed that maximum springiness obtained with interaction of levels of CR (X g) with UD (S) and SR (U). Similarly, minimum springiness was obtained at levels of interaction of CR (Y g) with UD (S) and SR (U). Increasing CR level, surprisingly increased springiness. This could be due to higher amount CR leading to highly concentrated form of acid and which could have contributed to produce CO_2 from $NaHCO_3$. According to Table 4.8, 4.9, 4.14 and 4.15, it can be inferred that the level of RE, UD, SR could be selected at Q, S, U parts with V parts of MPP and X g of CR and their combinations showed optimum springiness of 0.81±0.01 mm (T15), and it fell within the range established from market samples.

4.5.1.4 Cohesiveness

The force area of work during the second compression divided by the force area of work during the first compression determines cohesiveness. Cohesiveness is how well the product withstands a second deformation relative to its resistance under the first compression. Hence, the cohesiveness of product should be lower for easy biting. Cohesiveness was higher in low urad dhal portion added *idli* (Durgadevi and Shetty, 2012) as well as in unfermented batter (Kannan *et al.*, 2015).

As per Table 4.8, the maximum cohesiveness was ~0.9 obtained from RE, UD, SR, MPP and CR used at levels of P, R, T, V parts and X g (T1); Q, S, T, V parts and X g (T11) respectively, while the minimum hardness of ~2 was obtained from the combinations of RE, UD, SR, MPP and

CR at levels P, S, U, V parts and X g (T7), respectively. It is clearly explained in Annexure 1 that parts of SR and CR showed significantly effect ($p<0.05$) on cohesiveness of FMID individually except RE, UD and MPP and in almost all combinations showed non-significant ($p>0.05$) effect except SR and CR; and RE, UD and MPP. It can be observed from Table 4.9 that as the level of RE increased, the cohesiveness of samples increased (i.e., from 0.48 to 0.51) non-significantly ($p>0.05$), while as the level of UD, and SR increased, cohesiveness also decreased (i.e., from 0.51 to 0.48 and from 0.55 to 0.44, respectively) non-significantly ($p>0.05$). But as the level of CR increased, the cohesiveness of samples decreased (from 0.63 to 0.36) significantly ($p<0.05$). This could be due to the addition of CR which helps to produce CO_2 from $NaHCO_3$, leading to reduction in the internal strength i.e., cohesiveness.

Table 4.16: Effect of interaction of SR and CR on cohesiveness of FMID

Level of SR	**Level of CR**		**Overall Mean**
	X	**Y**	
T	0.741	0.376	0.559^{a}
U	0.529	0.350	0.440^{b}
Overall Mean	0.635^{x}	0.363^{y}	

FMID: Functional Multi-grain *Idli*

CD (SR) =0.075 CD (CR) =0.076 CD (SR*CR) =0.111

It can be observed from Table 4.16 that the least cohesiveness was obtained at the interaction level of SR (U) and CR (Y). The maximum cohesiveness was obtained when T parts of SR and X g of CR was used. According to the Annexure 1, level of RE, UD and MPP could be selected at P parts, S parts and V parts, respectively because of non-significant ($p>0.05$) effect of RE, UD and MPP (individually) on cohesiveness of FMID. As per Table 4.8, 4.9 and 4.16, SR and CR could be selected at levels U parts and Y g. The combination of selected levels of these ingredients i.e., RE (P), UD (R), SR (U), MPP (V) and CR (Y) resulted in the optimum level of cohesiveness on FMID, i.e., 0.37±0.02, Table 4.8, which also fell within the range established from market samples.

4.5.1.5 Chewiness

Chewiness is defined as the product of hardness, cohesiveness and springiness. It is expressed in Newtons/Joule. Low chewiness represents the softness of *idli*. As per Table 4.8, the maximum chewiness was ~4 obtained from RE, UD, SR, MPP and CR used at levels of P, R, T, V parts and X g (T1) and Q, S, T, V parts and X g (T11), respectively, while the minimum

chewiness of ~0.5 J was obtained from the combinations of RE, UD, SR, MPP and CR at levels Q, R, T, V parts and Y g, respectively (T25).

It is clearly explained in Annexure 1 that the parts of SR and CR showed significant effect ($p<0.05$) on chewiness of FMID individually except RE, UD and MPP, while some combinations i.e., SR and MPP; SR and CR; RE, UD and MPP; RE, UD, MPP and CR showed significant ($p<0.05$) effect on chewiness of FMID.

It can be observed from Table 4.9 that as the level of RE and MPP increased, the chewiness of samples increased (i.e., from 1.63 J to1.69 J and 1.65 J to1.68 J, respectively) non-significantly ($p>0.05$). On the other hand, as the level of UD, SR, and CR increased, chewiness decreased (i.e., from 1.83 J to 1.50 J, from 1.95 J to 1.37 J and from 2.48 J to 0.85 J, respectively) significantly ($p<0.05$). The high level of UD could be directly correlated with mucilagenous principle and high level of CR could be correlated with CO_2 production.

Table 4.17: Effect of interaction of SR with MPP and CR on chewiness of FMID

Level of SR	**Level of MPP**		**Level of CR**		**Overall Mean**
	V	**W**	**X**	**Y**	
T	2.176	1.741	3.062	0.855	1.959^{a}
U	1.128	1.628	1.902	0.854	1.378^{b}
Overall Mean	1.652^{x}	1.685^{x}	2.482^{p}	0.855^{q}	

FMID: Functional Multi-grain *Idli*

CD (SR) =0.363 CD (CR) =0.36 CD (MPP) =0.363

CD (SR * CR) =0.514 CD (SR * MPP) =0.514

It can be noted from Table 4.17 that least chewiness was obtained with U parts of SR with V parts of MPP; and U parts of SR with Y g of CR and maximum chewiness was obtained with T parts of SR with V parts of MPP; and T of parts SR with X g of CR. The increasing level of SR and CR decreased the chewiness on FMID significantly ($p<0.05$) from 1.95 J to 1.37 J and 2.48 J to 0.85 J, respectively. But increasing MPP level increased the chewiness of FMID from 1.65 J to 1.68 J non-significantly ($p>0.05$) due to the presence of high fiber content of MPP.

According to the Annexure1, it can be inferred that RE and UD could be selected at P and R parts because of non-significance effect ($p>0.05$) of these on chewiness. According to Table 4.8, 4.9 and 4.17, it can be inferred that the SR at U parts, MPP at V parts and CR at Y g could be used for preparation of optimum product because of the optimum levels of chewiness. Therefore, RE, UD could be selected at P and R parts respectively, SR at U parts, MPP at V parts

and CR at Y g considering chewiness of FMID. This combination level showed a chewiness value of 0.66±0.13 J which also fell within the range established from market samples (Table 4.8).

4.5.1.6 Resilience

Resilience is how well a product fights to regain its original height. It is calculated on the withdrawal of the first penetration, before the waiting period has started. Low resilience of *idli* shows that the *idli* is firmer and addition of 5 to 20% *Moringa oleifera* leaves in *idli* showed decreased level of resilience (Durgadevi and Shetty, 2012). As per Table 4.8, the maximum resilience was ~2.2 obtained from RE, UD, SR, MPP and CR used at levels of P, S, U, V parts and X g respectively (T7), while the minimum resilience of ~0.15 was obtained from the combinations of RE, UD, SR, MPP and CR at levels P, R, T, V parts and Y g (T17) and Q, S, U, W parts and Y g (T32).

As per Annexure 1 RE and CR showed significantly effect ($p<0.05$) on resilience individually, while UD, SR and MPP did not show any significant effect ($p>0.05$) on resilience. The significant effect was also observed in some combination of ingredients i.e., RE and CR; UD and SR; UD and MPP; RE, UD and SR; RE, SR and MPP; UD, SR and CR; UD, MPP and CR; RE, UD, MPP and CR; and RE, SR, MPP and CR showed significant effect ($p<0.05$) on resilience of FMID. It can be observed from Table 4.9 that as the level of RE and CR increased, the resilience of samples decreased (i.e., from 0.47 to 0.28, and 0.58 to 0.18, respectively) significantly ($p<0.05$),

Table 4.18: Effect of interaction of combination of UD with SR and CR on resilience of FMID

Level of UD	Level of SR		Level of MPP		Overall Mean
	T	U	V	W	
R	0.441	0.269	0.344	0.367	0.355^{a}
S	0.305	0.507	0.542	0.270	0.406^{a}
Overall Mean	0.373^{x}	0.388^{x}	0.443^{p}	0.316^{p}	

FMID: Functional Multi-grain *Idli*

CD (UD) =0.136 CD (SR) =0.136 CD (MPP) =0.136

CD (UD * SR) =0.192 CD (UD * MPP) =0.192

Table 4.19: Effect of interaction of RE with CR on resilience of FMID

Level of RE	Level of CR		Overall Mean
	X	Y	
P	0.776	0.179	0.478^{a}
Q	0.384	0.184	0.284^{b}
Overall Mean	0.58^{x}	0.182^{y}	

FMID: Functional Multi-grain *Idli*

CD (RE) =0.136 CD (CR) =0.136 CD (RE * CR) =0.192

while with increase in MPP, resilience decreased non-significantly ($p>0.05$) from 0.44 to 0.31. On the other hand, as the level of UD and SR increased, resilience increased (i.e., from 0.35 to 0.40 and from 0.37 to 0.38, respectively) non-significantly ($p>0.05$).

It can be noted from Table 4.18 that maximum resilience was obtained with interaction of S parts of UD and U parts of SR; and S parts of UD with V parts of MPP, while least resilience was obtained with R parts of UD and U parts of SR; and S parts of UD with W parts of MPP. It can be clearly observed from Table 4.19 that P parts of RE with V g of CR; and P parts of RE with Y g of CR showed maximum and minimum resilience of 0.776 and 0.179 respectively.

According to the Tables 4.8, 4.9, 4.18 and 4.19, that RE, UD, SR, MPP and CR could be selected at P, S, U, V parts and X g respectively, for optimum resilience on FMID. The combination of above-mentioned ingredients showed average resilience of 2.21±0.02 which also fells in the range of market samples (Table 4.8).

4.6 Ink Print Test

Ink print test denotes the number pores present per square meter area using graph paper. The higher number of pores indicates higher degree of softness of *idli* (Nazni and Shalini, 2010).

As per Table 4.8, the maximum number of pores of ~141 were obtained from RE, UD, SR, MPP and CR used at levels of Q, R, T, V parts and X g, respectively (T9), while the minimum number of pores were ~46 was obtained from the combinations of RE, UD, SR, MPP and CR used at P, S, U, V parts and Y g, respectively (T23). It is clearly revealed in Annexure 1 that parts of RE, UD, SR, MPP and CR showed significant effect ($p<0.05$) on number of pores of FMID individually and in almost all combinations expect, RE and SR; RE and CR; and UD and CR. It can be observed from Table 4.9 that as the level of UD, SR, MPP and CR increased, the number of pores of samples decreased (i.e., from 94.646 to 90.167, from 95.00 to 89.813, from 93.917 to 90.896 and 97.229 to 87.583, respectively) significantly ($p<0.05$), while as the level of RE increased, the number of pores also increased (i.e., from 89.625 to 95.188) significantly ($p<0.05$). This could be correlated to the reported results of Durgadevi and Shetty (2014) and Sowbhagya *et al.,* (1991) who stated that high amylose or intermediate amylose variety leads to the production soft *idli*.

It can be inferred from Table 4.20 that maximum number of pores were obtained at combinations of Q parts of RE and R parts of UD; and Q parts of RE and V parts of MPP, while minimum number of pores were obtained at combinations of P and R parts of RE and UD respectively; and P parts of RE and V parts of MPP. It is clear from Table 4.21 that the interaction

of UD with SR and MPP separately yielded maximum pores at P and R parts of RE and UD respectively; and S parts of UD with W parts of MPP (~99 and ~96 respectively) on FMID. The minimum number of pores were obtained with S and U parts of UD and SR respectively; and S parts of UD with W parts of MPP (~89 and ~85 respectively) on FMID.

Table 4.20: Effect of interaction of combination of RE with UD and CR on number of pores of FMID

Level of RE	Level of UD		Level of MPP		Overall Mean
	R	S	V	W	
P	88.583	90.667	88.500	90.750	89.625^{b}
Q	100.708	89.667	99.333	91.042	95.188^{a}
Overall Mean	94.646^{x}	90.167^{y}	93.917^{p}	90.896^{q}	

FMID: Functional Multi-grain *Idli*

CD (RE) =0.782 CD (UD) =0.782 CD (MPP) =0.782

CD (RE * UD) =1.107 CD (RE* MPP) =1.107

Table 4.21: Effect of interaction of combination of UD with SR and MPP on number of pores of FMID

Level of UD	Level of SR		Level of MPP		Overall Mean
	T	U	V	W	
R	99.042	90.250	93.125	96.167	94.646^{a}
S	90.958	89.375	94.708	85.625	90.167^{b}
Overall Mean	95^{x}	90.813^{y}	93.917^{p}	90.896^{q}	

FMID: Functional Multi-grain *Idli*

CD (UD) =0.782 CD (SR) =0.782 CD (MPP) =0.782

CD (UD * SR) =1.107 CD (UD* MPP) =1.107

Table 4.22: Effect of interaction of combination of SR with MPP and CR on number of pores of FMID

Level of SR	Level of MPP		Level of CR		Overall Mean
	V	W	X	Y	
T	99.125	90.875	100.583	89.417	95.000^{a}
U	88.708	90.917	93.875	85.750	89.812^{b}
Overall Mean	93.917^{x}	90.896^{y}	97.229^{p}	87.583^{q}	

FMID: Functional Multi-grain *Idli*

CD (SR) =0.782 CD (MPP) =0.782 CD (CR) =0.782

CD (SR * MPP) =1.107 CD (SR * CR) =1.107

It can be clearly observed from Table 4.22 that maximum number of pores (~100) were obtained with a combination T parts of SR with V parts of MPP and X g of CR separately; while minimum number of pores (~88) were obtained with a combination of U parts of SR and V parts of MPP; and U parts of SR with Y g of CR respectively. It can be observed from Table 4.23.

Table 4.23: Effect of interaction of MPP and CR on number of pores of FMID

Level of MPP	Level of CR		Overall Mean
	X	Y	
V	101.292	86.542	93.917[a]
W	93.167	88.625	90.896[b]
Overall Mean	97.230[x]	87.585 [y]	

FMID: Functional Multi-grain *Idli*

CD (MPP) =0.782 CD (CR) =0.782 CD (MPP * CR) =1.107

that maximum number of pores (~101) were obtained with a combination of V parts of MPP and X g of CR and minimum number of pores (~86) were obtained in combinations with V parts of MPP with Y g CR. Hence, according to Tables 4.8, 4.9, 4.20, 4.21, 4.22 and 4.23 RE, UD, SR, MPP and CR at Q, R, T, V parts and Y g, respectively could be selected for optimum number of pores in FMID. The selected combination of FMID showed number of pores i.e., 141±1.15 which also fell within the range established from market samples (Table 4.8).

4.7 Optimization based on sensorial attributes

The effect of different levels of RE, UD, SR, MPP and CR on the descriptive analysis for describing the sensory attributes of FMID like color, dryness of interior, porosity, firmness, springiness, fermented aroma, acidic taste and overall acceptability are discussed in following sub sections.

4.7.1 Color

Surface color (brown to white) of food is the most important subjective quality parameter observed by consumers. It is the first impression about food will decides the acceptance or rejection of food (Leon *et al.,* 2006). Nazni and Shalini (2010) have found that replacing certain part of rice with sorghum in *idli* showed higher score (~7.8) than complete replacement of rice (~7). As per Table 4.24 (a), the maximum colour score was 7.086 obtained from RE, UD, SR, MPP and CR used at levels of Q, R, U, V parts and Y g, respectively (T29), while the minimum colour score was 0.229 obtained from the combinations of P, R and U parts of RE, UD and SR, W parts of MPP and Y g CR (T2). It is clearly explained from Annexure 2 that all i.e. RE, UD, MPP and CR (except SR) showed significant effect ($p<0.05$) on colour of FMID individually and in some combinations i.e. RE and UD; RE and CR; UD and SR; UD and CR; SR and CR; MPP and CR; SR, MPP and CR; RE, UD, SR and CR. It can be observed from Table 4.26 that as the level of RE, UD, SR and CR increased, the colour scores of samples increased (i.e., from 4.14 to 5.02; from 4.20 to 4.96; from 4.39 to 4.77 and from 3.98 to 5.18, respectively), while as the level

Table 4.24 (a) Effect of different level of RE, UD, SR, MPP and CR on sensorial attributes of FMID

Treatment	Parts				CR(g)	Color	Dryness of interior	Porosity	Firmness
	RE	UD	SR	MPP					
T1	P	R	T	V	X	1.029±0.331	5.386±0.761	4.586±0.792	7.300±0.491
T2	P	R	T	W	X	0.229±0.211	5.486±0.931	5.943±0.532	6.714±0.521
T3	P	S	T	V	X	5.157±0.911	5.657±1.101	6.529±0.602	5.500±0.921
T4	P	S	T	W	X	3.629±0.911	5.114±0.931	4.829±0.642	5.529±0.601
T5	P	R	U	V	X	3.786±0.491	5.786±0.931	4.671±0.622	4.843±0.521
T6	P	R	U	W	X	3.543±0.961	5.929±0.991	5.000±0.492	5.400±0.492
T7	P	S	U	V	X	4.429±0.571	4.671±0.731	5.786±0.532	4.429±0.872
T8	P	S	U	W	X	4.143±0.491	4.914±0.861	6.043±0.602	5.343±0.842
T9	Q	R	T	V	X	4.100±0.621	4.857±0.421	5.543±0.352	4.157±0.662
T10	Q	R	T	W	X	3.486±0.591	3.886±0.491	5.514±0.432	3.371±0.602
T11	Q	S	T	V	X	6.071±0.771	5.029±0.271	5.286±0.592	3.757±0.712
T12	Q	S	T	W	X	4.071±0.541	5.486±0.331	5.114±0.612	4.657±0.672
T13	Q	R	U	V	X	4.271±0.721	4.186±0.681	5.971±0.442	3.686±0.862
T14	Q	R	U	W	X	6.243±0.721	3.700±0.871	6.514±0.562	4.829±1.112
T15	Q	S	U	V	X	5.686±0.841	4.986±0.551	5.700±0.722	4.429±0.752
T16	Q	S	U	W	X	3.843±0.601	5.271±0.621	5.271±0.821	3.786±0.472
T17	P	R	T	V	Y	5.957±0.691	4.743±0.611	2.386±0.391	4.186±0.902
T18	P	R	T	W	Y	4.571±0.441	5.257±0.481	4.600±0.731	3.314±0.332
T19	P	S	T	V	Y	6.357±0.771	4.443±0.891	6.286±0.711	3.543±0.712
T20	P	S	T	W	Y	4.614±0.691	3.700±0.530	5.314±0.371	4.029±0.772
T21	P	R	U	V	Y	5.800±0.421	3.686±0.522	5.300±0.741	2.714±0.921
T22	P	R	U	W	Y	2.814±0.571	2.586±0.712	4.400±0.671	4.171±1.01
T23	P	S	U	V	Y	5.757±0.511	4.571±0.491	5.457±0.651	5.343±0.531
T24	P	S	U	W	Y	4.414±0.711	4.514±0.951	5.486±0.541	4.071±0.961
T25	Q	R	T	V	Y	5.043±0.561	4.629±0.651	6.071±0.691	3.914±0.961
T26	Q	R	T	W	Y	4.486±0.441	4.086±0.431	5.814±0.531	3.671±0.551
T27	Q	S	T	V	Y	6.357±0.481	4.200±0.611	5.671±0.821	2.771±0.701
T28	Q	S	T	W	Y	5.514±0.901	4.271±0.531	6.014±0.531	2.457±0.641
T29	Q	R	U	V	Y	7.086±0.591	4.400±0.651	5.414±0.851	3.457±0.561
T30	Q	R	U	W	Y	4.700±0.711	4.557±0.701	4.886±0.651	3.314±0.661
T31	Q	S	U	V	Y	5.900±0.931	4.743±0.811	4.214±0.791	5.257±0.872
T32	Q	S	U	W	Y	3.929±0.611	5.843±0.781	4.800±0.971	4.143±0.822

FMID: Functional Multi-grain *Idli*

Color CD (RE*UD*SR*MPP*CR) (P<0.05) = 2.740; Porosity CD (RE*UD*SR*MPP*CR) (P<0.05) =2.735

Dryness of interior CD (RE*UD*SR*MPP*CR) (P<0.05) = 3.027;

Table 4.25 (b): Effect of different level of RE, UD, SR, MPP and CR on sensorial attributes of FMID

Treatment	Parts				CR (g)	Springiness	Fermented aroma	Acidic taste	overall acceptability
	RE	UD	SR	MPP					
T1	P	R	T	V	X	4.129±1.100	3.257±0.871	2.514±1.001	6.400±0.531
T2	P	R	T	W	X	5.071±1.001	2.586±0.751	2.029±0.731	4.914±0.561
T3	P	S	T	V	X	3.829±1.011	4.014±1.041	2.471±0.791	6.029±0.671
T4	P	S	T	W	X	4.086±1.001	4.643±1.021	4.114±0.861	6.457±0.581
T5	P	R	U	V	X	4.757±0.682	3.357±0.781	3.000±0.791	6.114±0.581
T6	P	R	U	W	X	5.500±0.832	2.814±0.750	2.443±0.721	5.514±0.711
T7	P	S	U	V	X	5.814±0.732	4.514±0.970	3.200±0.921	8.071±0.241
T8	P	S	U	W	X	5.771±0.682	4.671±0.950	3.786±1.011	8.271±0.441
T9	Q	R	T	V	X	5.071±0.812	4.771±0.901	4.200±0.851	6.729±0.841
T10	Q	R	T	W	X	6.314±0.801	4.314±1.001	4.171±1.00	6.700±0.961
T11	Q	S	T	V	X	6.171±0.701	3.871±0.861	3.671±0.881	7.214±0.570
T12	Q	S	T	W	X	4.557±0.912	4.700±0.981	4.414±1.001	6.700±0.960
T13	Q	R	U	V	X	6.286±0.612	4.100±0.641	3.014±0.981	7.557±0.820
T14	Q	R	U	W	X	6.900±0.602	2.571±0.671	2.486±0.971	6.414±0.580
T15	Q	S	U	V	X	4.486±0.552	3.529±0.570	3.571±0.741	7.057±0.660
T16	Q	S	U	W	X	6.057±0.692	5.543±0.710	5.257±0.961	6.186±0.410
T17	P	R	T	V	Y	5.900±0.702	3.100±0.880	3.043±0.991	5.257±0.590
T18	P	R	T	W	Y	7.100±1.012	3.757±0.710	3.429±0.721	7.457±0.410
T19	P	S	T	V	Y	7.629±0.882	5.457±0.980	4.600±0.821	8.429±0.451
T20	P	S	T	W	Y	4.357±0.902	4.086±1.020	3.071±0.901	6.786±0.851
T21	P	R	U	V	Y	4.643±0.652	5.357±0.850	4.086±0.961	8.314±0.611
T22	P	R	U	W	Y	5.186±1.011	4.414±0.970	3.100±0.881	5.986±0.831
T23	P	S	U	V	Y	4.243±0.541	3.700±1.010	2.829±0.821	7.871±0.361
T24	P	S	U	W	Y	4.871±0.561	3.186±0.890	2.643±0.701	7.314±0.591
T25	Q	R	T	V	Y	6.043±0.731	4.057±1.000	3.286±1.021	8.614±0.431
T26	Q	R	T	W	Y	5.529±0.711	4.243±0.860	3.614±0.701	6.900±0.771
T27	Q	S	T	V	Y	6.243±0.611	6.186±1.391	3.700±0.961	9.386±0.271
T28	Q	S	T	W	Y	6.671±0.391	4.371±0.681	3.714±0.721	7.686±0.641
T29	Q	R	U	V	Y	5.100±1.051	5.214±0.981	4.300±0.991	8.114±0.711
T30	Q	R	U	W	Y	6.057±0.711	4.657±0.651	4.143±0.611	7.386±0.481
T31	Q	S	U	V	Y	4.757±1.031	3.786±0.881	2.957±0.901	6.143±0.641
T32	Q	S	U	W	Y	4.971±0.921	4.200±0.960	3.800±0.84	6.500±0.75

FMID: Functional Multi-grain *Idli*

Springiness CD (RE*UD*SR*MPP*CR) (P<0.05) = 3.774; Acidic taste CD (RE*UD*SR*MPP*CR) (P<0.05) = 3.741
Fermented aroma CD (RE*UD*SR*MPP*CR) (P<0.05) = 3.823; Firmness CD (RE*UD*SR*MPP*CR) (P<0.05) = 3.184
Overall acceptability CD (RE*UD*SR*MPP*CR) (P<0.05) = 2.000

Table 4.26: Effect of different ingredient on Sensory parameters of FMID individually

Ingredient	Level	Color	Dryness of interior	Porosity	Firmness	Springiness	Fermented aroma	Acidic taste	Overall acceptability
RE	P	4.140^{x}	4.780^{x}	5.160^{x}	4.780^{x}	5.180^{x}	3.930^{x}	3.150^{x}	6.880^{x}
	Q	5.020^{y}	4.660^{x}	5.490^{x}	3.950^{y}	5.660^{x}	4.360^{x}	3.740^{x}	7.200^{x}
UD	R	4.200^{p}	4.570^{p}	5.160^{p}	4.320^{p}	5.600^{p}	3.910^{p}	3.300^{p}	6.770^{p}
	S	4.960^{q}	4.860^{p}	5.490^{p}	4.410^{p}	5.240^{p}	4.380^{p}	3.590^{p}	7.310^{q}
SR	T	4.390^{l}	4.790^{l}	5.350^{l}	4.400^{l}	5.500^{l}	4.190^{l}	3.480^{l}	7.040^{l}
	U	4.770^{l}	4.650^{l}	5.310^{l}	4.330^{l}	5.340^{l}	4.100^{l}	3.410^{l}	7.050^{l}
MPP	V	5.170^{h}	4.750^{g}	5.300^{g}	4.330^{g}	5.320^{g}	4.270^{g}	3.400^{g}	7.330^{g}
	W	3.990^{g}	4.690^{g}	5.350^{g}	4.390^{g}	5.520^{g}	4.030^{g}	3.490^{g}	6.770^{h}
CR (g)	X	3.980^{a}	5.020^{a}	5.520^{a}	4.860^{a}	5.300^{a}	3.950^{a}	3.400^{a}	6.650^{a}
	Y	5.180^{b}	4.410^{b}	5.140^{a}	3.860^{b}	5.540^{a}	4.340^{a}	3.490^{a}	7.440^{b}

FMID: Functional Multi-grain *Idli*

CD (Color)= 0.66 CD (Dryness of interior) = 0.79 CD (Porosity)= 0.59
CD (Firmness)=0.63 CD (Springiness)= 0.78 CD (Fermented aroma) = 0.69
CD (Acidic taste)=0.68 CD (Overall acceptability)= 0.58

Table 4.27: Effect of interaction of combination of CR with RE, UD and CR on color of FMID

Level of CR	Level of RE		Level of UD		Levels of SR		Level of MPP		Overall Mean
	P	Q	R	S	T	U	V	W	
X	3.243	4.721	3.336	4.629	3.471	4.493	4.316	3.548	3.970^{b}
Y	5.046	5.318	5.057	5.629	5.304	5.05	6.032	4.321	5.220^{a}
Overall Mean	4.145^{y}	5.020^{x}	4.197^{q}	5.129^{p}	4.388^{l}	4.772^{l}	5.174^{g}	3.935^{h}	

FMID: Functional Multi-grain *Idli*

CD (RE) =0.45 CD (UD) =0.45 CD (SR) =0.45
CD (MPP) =0.45 CD (CR) =0.45 CD (SR * MPP) =0.63
CD (SR * CR) =0.63

of MPP increased, colour score decreased, this could be due to the color of MPP. The effect of interaction of CR with RE, UD, SR and MPP on color of FMID can be observed from Table 4.27. The maximum scores were obtained from the levels of 100 g of CR with Q parts of RE, S parts of UD, T parts of SR and V parts of MPP separately (i.e., 5.318; 5.629; 5.304 and 6.032, respectively),

while minimum scores were obtained at levels of interaction of CR being X g with P parts of RE, R parts of UD, T parts of SR and W parts of MPP (i.e., 3.243, 3.336, 3.347 and 3.548, respectively).

Table 4.28: Effect of interaction of combination of UD with RE and SR on color of FMID

Level of UD	Level of RE		Levels of SR		Overall Mean
	P	Q	T	U	
R	3.466	4.927	3.613	5.163	4.292^{a}
S	4.813	5.113	4.78	4.763	4.867^{a}
Overall Mean	4.140^{x}	5.02^{x}	4.197^{q}	4.963^{p}	

FMID: Functional Multi-grain *Idli*

CD (RE) =0.45 CD (UD) =0.45 CD (SR) =0.45

CD (CR) =0.45 CD (MPP) =0.45 CD (SR * MPP) =0.63

CD (SR * Curd) =0.63

Table 4.28 shows the interaction of UD with RE and SR on color of FMID. The maximum scores obtained from the interaction of S parts of UD with Q parts of RE and U parts SR (i.e., 5.113 and 5.163, respectively). while, minimum scores were obtained from interaction R parts of UD with P parts of RE and T parts of SR separately (i.e., 3.466 and 3.613, respectively).

According to the Table 4.24 (a), 4.26, 4.27 and 4.28, it can be inferred that the combination of RE, UD, SR at Q, S, U parts level respectively and V parts of MPP with Y g of CR could be selected for obtaining optimum colour score of 5.900±0.931 [Table 4.24 (a)] for present research, which also fell within the range established from market samples.

4.7.2 Dryness of interior

Dryness of interior represents the dryness of product's interior. This could be due to the hydroscopic nature of food. As per Table 4.24 (a), the maximum score (i.e., 5.929) was obtained from the combination of RE, UD, SR, MPP and CR at levels of P, R, U, W parts and X g (T6) respectively, while minimum score (i.e., 2.586) was obtained from combination of RE, UD, SR, MPP and CR at levels of P, R, U, W parts and Y g (T22), respectively. It can be clearly noted from Annexure 2 that CR solely showed significant ($p<0.05$) effect on dryness of interior, while other ingredients i.e., RE, UD, SR and MPP showed no significant effect ($p>0.05$) on dryness of interiors. Further, only the interaction of RE and CR showed significant effect ($p<0.05$), while other combinations showed non-significant ($p>0.05$) on dryness of interiors of *idli*. It can be inferred from the Table 4.26, that as the levels of RE, SR and MPP increased, the scores of dryness of interior decreased (i.e., from 4.780 to 4.660, from 4.790 to 4.650, and from 4.750 to 4.690, respectively) non-significantly ($p>0.05$) individually, but with increase in the levels of UD, the

scores increased non-significantly (p>0.05). On the other hand, as level of CR increased, the scores of dryness of interior decreased (i.e., from 5.020 to 4.410) significantly (p<0.05).

Table 4.29: Effect of interaction of RE and CR on dryness interior of FMID

Level of RE	Level of CR		Overall Mean
	X	Y	
P	5.368	4.188	4.778[a]
Q	4.675	4.636	4.655[a]
Overall Mean	5.021[x]	4.412[x]	

FMID: Functional Multi-grain *Idli*

CD (RE) =0.495 CD (CR) =0.495 CD (RE * CR) =0.701

It can be clearly observed from Table 4.29 that maximum score (i.e., 5.368) and minimum score of FMID (4.188) for dryness of interior of FMID was obtained from interaction of P parts RE with X g of CR; and P parts RE with Y g CR, respectively. According to Tables 4.24, 4.26 and 4.29, the levels of RE and CR could be selected at levels of Q parts and Y g for optimum dryness of interiors. Other ingredients i.e., UD, SR and MPP showed non-significant effect (p>0.05) individually and with all other combination. Therefore, low level of UD (R parts), high level of SR (U parts) and MPP (W parts) could be selected for dryness of interior parameter and the combination of RE (Q), UD (R), SR (U), MPP (W) and CR (Y g) showed mean score of 4.557±0.70 [Table 4.24 (a)] which also fell within the range established from market samples.

4.7.3 Porosity

Sucheelamma and Rao (1974) explained that the porous nature of *idli* is due to surface active protein and arabinogalactan in urad dhal. Kannanan *et al.*, (2015) noted that increasing fermentation time in batter produced highly porous *idli*, which was found under scanning electron microscopy. Though, Ghasemi *et al.,* (2009) noted that steam decreases pore size and porosity of cooked rice grain.

Table 4.30: Effect of interaction of RE and CR on porosity of FMID

Level of RE	Level of UD		Overall Mean
	R	S	
P	4.611	5.716	5.163[a]
Q	5.716	5.266	5.491[a]
Overall Mean	5.163[x]	5.491[x]	

FMID: Functional Multi-grain *Idli*

CD (RE) =0.447 CD (UD) =0.447 CD (RE * UD) =0.633

As per Table 4.24 (a), the maximum porosity score (i.e., 6.529) was obtained from the combination of P parts of RE, S parts of UD, T parts of SR, V parts of MPP and X g of CR (T3), while minimum score (i.e., 2.386) for porosity of FMID was obtained from the combination of P, R and T parts of RE, UD and SR respectively; V parts of MPP and Y g of CR (T17).

As per Annexure 2, the levels of RE, UD, SR, MPP and CR showing non-significant effect ($p>0.05$) on FMID individually, while interaction of RE and UD; RE, SR and CR; RE, UD, SR and MPP showed significant effect ($p<0.05$) on porosity of FMID. It is evident from Table 4.30, that maximum score for porosity (i.e., 5.716) was obtained with combination of P parts of RE and S parts to UD; and vice versa i.e., Q parts of RE with R parts of UD; while the minimum score (i.e., 4.611) was obtained from a combination of P and R parts each of RE with UD.

According to the Table 4.25 (b) and 4.30, it can be inferred that RE and UD at level of P and S parts, respectively could be selected for optimum porosity of FMID. SR, MPP and CR showed significant effect ($p<0.05$) with some combinations. Therefore, their levels could be fixed as U parts, W parts and Y g and combination of RE (P), UD (S), SR (U), MPP (W) and CR (Y) scored porosity of 5.486±0.541 [Table 4.24 (a)] which also fell within the range established from market samples.

4.7.4 Firmness

Firmness represents the hardness of food. According to Table 4.24 (a), that the maximum score (i.e., 7.300) was obtained with P, R, T parts of RE, UD and SR respectively, V parts of MPP and X g of CR (T1) and minimum score on firmness (i.e., 2.457) of FMID was obtained from RE, UD, SR, MPP and CR at levels of Q, S, T, W parts and Y g (T28) respectively. It can be observed from Annexure 2 that the RE and CR showed significant ($p<0.05$) effect on firmness of FMID individually and in their combination; besides the combination of UD, SR and MPP.

Table 4.31: Effect of interaction of RE and CR on firmness of FMID

Level of RE	**Level of CR**		**Overall Mean**
	X	**Y**	
P	5.632	3.921	4.777^{a}
Q	4.084	3.805	3.945^{b}
Overall Mean	4.858^{x}	3.863^{y}	

FMID: Functional Multi-grain *Idli*

CD (RE) =0.527 CD (CR) =0.527 CD (RE * CR) =0.737

It can be clearly observed from the Table 4.26 that as the level of RE and CR increased, the firmness of samples decreased (i.e., from 4.780 to 3.950 and from 4.860 to 3.860, respectively)

significantly ($p<0.05$), but as the level of SR increased, firmness of sample decreased (i.e., from 4.400 to 4.330) non-significantly ($p>0.05$). As the level of UD increased, firmness also increased (i.e., from 4.320 to 4.410 and from 4.330 to 4.390) non-significantly ($p>0.05$). It is evident from Table 4.31 that maximum score for firmness (i.e., 5.632) was obtained at levels of P parts of RE with X g of CR and minimum score for firmness (i.e., 3.805) was obtained at levels of Q parts of RE with Y g of CR. Therefore, it can be inferred from Tables 4.25, 4.26 and 4.31 that the levels of RE and CR could be selected at Q parts and Y g, respectively, while SR, UD and MPP could be selected at U, S and W parts because of non-significant effect ($p>0.05$) on firmness of FMID and with the aim of increasing the amount of SR and MPP in optimized product. The combination of RE (Q), UD (S), SR (U), MPP (W) and CR (Y g) of ingredients showed mean firmness of 4.143±0.821 [Table 4.24 (a)] which also fell in the range of values obtained with market samples.

4.7.5 Springiness

Springiness is measured by placing the food sample between palate and teeth measuring the quickness of food recovery while removing force from food. As per Table 4.25 (b), that maximum score for springiness (i.e., 7.629) was obtained from a combination of RE, UD, SR, MPP and CR at levels of P, S, T, V parts and Y g (T19) and minimum score (i.e., 3.829) was obtained with the corresponding values of these ingredients at P, S, U, V parts and X g (T3).

It can be observed from Annexure 2, that all ingredients (RE, UD, SR, MPP and CR) individually and in combination showed non-significant ($p>0.05$) effect on springiness of FMID, except the combination of SR with CR which showed significant ($p<0.05$) effect.

Table 4.32: Effect of interaction of RE with UD on springiness of FMID

Level of SR	**Levels of CR**		**Overall Mean**
	X	**Y**	
T	4.904	6.105	5.504^{a}
U	5.696	4.979	5.338^{a}
Overall Mean	5.300^{x}	5.542^{x}	

FMID: Functional Multi-grain *Idli*

CD (SR) =0.563 CD (CR) =0.563 CD (SR * CR) =0.765

It can be observed from Table 4.26 that as the level of RE, MPP and CR increased, springiness increased (i.e., from 5.180 to 5.660, from 5.320 to 5.520 and from 5.300 to 5.540, respectively) non-significantly ($p>0.05$), while as increase in level of UD and SR, springiness decreased (i.e., from 5.600 to 5.240 and from 5.500 to 5.340, respectively) non-significantly

(p>0.05). As per Table 4.32, the maximum score for springiness (i.e., 6.105) was obtained from T parts of SR with Y g of CR and minimum score (i.e., 4.904) was obtained with T parts SR and X g CR.

According to the Table 4.25 (b), 4.26 and 4.32, it can be inferred that SR and CR could be selected at a level of T parts and Y g, respectively for optimum springiness. Other ingredients can be selected at level of P and R parts of RE and UD, respectively and W parts of MPP so as to increase the percentage of SR and MPP in the optimized combination and also on account of non-significant effect (p>0.05) of these ingredients on springiness of FMID and the combination of selected ingredients showed score of 5.186±1.011 [Table 4.25 (b)] which also fells in the range of market samples.

4.7.6 Fermented aroma

Agarwal *et al.,* (2000) noted ketones, diols and acids as a flavor profile of fermented *idli* Lactic acid bacteria and desirable yeast causes an improved fermented flavor. In our research which was achieved by addition of sour curd while preparing batter for FMID. As per Table 4.25 (b) that Maximum score (i.e., 6.186) on fermented aroma of *idli* obtained from RE, UD, SR, MPP and CR at levels of Q, S, T, V parts, and Y g (T27) respectively, and minimum score (2.586) was obtained from RE, UD, SR, MPP and CR at levels of P, R, T, W parts and X g (T2) respectively. It can be inferred from Annexure 2 that the UD, SR and CR combination showed significant effect (p<0.05) on fermented aroma of FMID. But all ingredients (RE, UD, SR, MPP and CR) individually and all other combinations showed non-significant effect (p>0.05) on fermented aroma. But based on Annexure 2, that level of MPP (V parts) and RE (Q parts) selected because of non-significant effect on fermented aroma individually and in combination. Hence, UD, SR and CR selected at level of S, T parts and Y g respectively. The combination of Q parts of RE, S parts of UD, T parts of SR, V parts of MPP and Y g of CR showed optimum for fermented aroma from Table 4.25 (b) (6.186±1.391) (T27), and which also fells in the range of market samples.

4.7.7 Acidic taste

The acceptable acidity in *idli* is reported to be ≥1% (as lactic acid). The higher increase in acidity leads to sour unacceptable taste and hardness of *idli* because of over fermentation (Sonawane *et al.,* 2019). The maximum (4.600) and minimum (2.029) mean scores for acidic taste were obtained for combination of P parts RE, S parts UD, T parts SR, V parts MPP and Y g CR and P parts RE, R parts UD, T parts SR, W parts MPP and X g CR, respectively [Table 4.25 (b)].

As per Annexure 2, all ingredients (RE, UD, SR MPP and CR) showed non-significant effect ($p>0.05$) on acidic taste of FMID both individually and in combination. Therefore, based on over all acceptability the combination that could be selected from Table 4.25 (b) is P parts of RE, S parts of UD, T parts of SR, V parts of MPP and Y g of CR which showed optimum acidic taste on FMID, i.e., 4.600±0.821, Table 4.25 (b) (T19).

4.7.8 Overall acceptability

As per Table 4.25 (b), it can be observed that maximum score for overall acceptability (i.e., 9.386) was obtained from P parts of RE, S parts of UD, T parts of SR, V parts of MPP and Y g of CR (T27) and minimum score (i.e., 4.914) was obtained from P parts of RE, R parts of UD, T parts of SR, W parts of MPP and X g of CR (T2). It can be observed from Annexure 2 that UD, MPP and CR showed significant effect ($p<0.05$) on overall acceptability both individually and in some combinations. However, RE and CR did not show significant effect ($p>0.05$) both individually and interaction with other ingredients. It can be observed from Table 4.26 that as the level of RE and SR increased, the scores for overall acceptability increased non-significantly ($p>0.05$) (i.e., from 6.880 to 7.200, from 7.040 to 7.050, respectively), while with increase in level of UD and CR, overall acceptability score increased significantly ($p<0.05$) (i.e., from 6.770 to 7.310, and from 6.650 to 7.440, respectively) on FMID.

As per Table 4.33, the maximum score was obtained from RE with UD at levels of P and S parts (i.e., 7.523) and RE with SR at levels of Q and T parts (i.e., 7.488) respectively, while minimum score was obtained from RE with UD at levels P and R parts (i.e., 6.586) and RE with SR at levels of P and U parts.

Table 4.33: Effect of interaction RE with UD and SR on over all acceptability of FMID

Level of RE	Level of UD		Levels of SR		Overall Mean
	R	S	T	U	
P	6.245	7.523	6.586	7.182	6.884^{a}
Q	7.302	7.105	7.488	6.920	7.204^{a}
Overall Mean	6.774^{x}	7.314^{x}	7.037^{p}	7.051^{p}	

FMID: Functional Multi-grain *Idli*

CD (RE) =0.437 CD (UD) =0.437 CD (SR) =0.437

CD (RE * UD) =0.618 CD (RE * SR) =0.618

It can be observed from Table 4.34 that maximum score (i.e., 7.680) was obtained from SR with CR at levels of T parts and Y g respectively, respectively and minimum score (i.e., 6.393) was obtained from T parts of SR with X g of CR on FMID. Therefore, as per Table 4.26, 4.33 and 4.34 that it could be inferred that Q parts RE, S parts UD, T parts SR, V parts MPP and Y g CR

could be selected as optimum i.e., 8.429±0.45 combination [Table 4.25(b)] which also fell in the range of market samples.

Table 4.34: Effect of interaction SR with CR on over all acceptability of FMID

Level of SR	Level of CR		Overall Mean
	X	Y	
T	6.393	7.680	7.036^{a}
U	6.898	7.204	7.051^{b}
Overall Mean	6.6455^{y}	7.442^{x}	

FMID: Functional Multi-grain *Idli*

CD (SR) =0.437 CD (CR) =0.437 CD (SR * CR) =0.618

4.8 Selection of ingredients based on Sensory and Textural parameters

The discussion held between 4.1.1 and Table 4.35 on the descriptive sensory analysis of FMID resulted to the combination of RE- Q parts, UD-S parts, SR- T parts, MPP- V parts and CR- Y g for final optimization of ingredients required for FMIIM as most of the parameters showed the optimum value for this combination. According to the TPA and ink print test RE-Q parts, UD-S parts, SR- U parts, MPP- V parts and CR- X g showed optimum results. The combination selected from descriptive sensory analysis results and discussion (section 4.5) was compared with the combination chosen for each parameter of TPA. From Table 4.8, it can be observed that there was non-significant ($p>0.05$) difference between values of hardness (3.190±0.020), springiness (0.740±0.010), cohesiveness (0.360±0.020), chewiness (0.870±0.060) and resilience (0.190±0.010) at RE- Q parts, UD-S parts, SR- U parts, MPP- V parts and CR- Y g with respect to hardness (3.310±0.130 N), springiness (0.660±0.010 mm), cohesiveness (0.380±0.020), chewiness (0.850±0.070 J) and resilience (0.200±0.010) at RE- Q parts, UD-S parts, SR- T parts, MPP- V parts and CR- Y g. However, RE (P), UD (S), SR (U), MPP (W) and CR (Y) scored porosity of 5.486±0.541 and there was significant different ($p<0.05$) between adhesiveness (-0.002±0.00) and number of pores (83.670±0.670) for RE- Q parts, UD-S parts, SR- U parts, MPP- V parts and CR- Y g with that of respective values of adhesiveness (-0.016±0.010) and number of pores (111±0.58) for RE- Q parts, UD-S parts, SR- T parts, MPP- V parts and CR- Y g. Hence, RE- Q parts, UD-S parts, SR- T parts, MPP- V parts and CR- Y g was selected as an optimum combination from the results and discussion made from section 4.1 to Table 4.35, which was used for the preparation of FMIIDM as it showed optimum or higher values for all the parameters in comparison to range established from market samples.

Table 4.35: Optimization level of ingredients with respect to different parameters

S.No	Parameter	Combination of ingredients required to prepare FMIIM					Values (Sensory, n=7) (TPA=3) (IPT =3)
		Parts					
		RE	UD	SR	MPP	CR(g)	
1	Hardness (N)	Q	S	U	W	Y	3.330±0.270
2	Adhesiveness (N.s)	Q	S	U	V	X	-0.003±0.001
3	Springiness(mm)	Q	S	U	V	X	0.810±0.010
4	Cohesiveness	P	R	U	V	Y	0.370±0.020
5	Chewiness (J)	P	R	U	V	Y	0.560±0.130
6	Resilience	P	S	U	V	X	2.21±0.02
7	No. of pores	Q	R	T	V	X	141±1.150
8	Color	Q	S	U	V	Y	5.900±0.093
9	Dryness of interior	Q	R	U	W	Y	4.557±0.701
10	Porosity	P	S	U	W	Y	5.486±0.541
11	Firmness	Q	R	T	V	Y	3.914±0.961
12	Springiness	P	R	T	W	Y	7.100±1.012
13	Fermented aroma	Q	S	T	V	Y	6.186±1.391
14	Acidic taste	P	R	T	V	Y	3.429±0.721
15	Overall acceptability	Q	S	T	V	Y	9.386±0.271

n: number of samples

4.9 Proximate composition of optimized Functional Multi-Grain Instant *Idli* Mix (FMIIDM), control mix (CM)

The optimized functional multi-grain instant *idli* mix was prepared using RE, UD, SR and MPP as the major ingredients. The two different controls were prepared i.e., 1) CM1: without both MPP and SR (and contained RE-Q parts, UD-S parts and CR- Y g with adjunct ingredients 2) CM2: without MPP, but containing SR (RE -U parts, UD- S parts, SR- T parts, CR-Y g with adjunct ingredients). The optimized sample was compared with the two controls for physico-chemical, bio-functional, textural properties and microbial qualities. Thus, three different mixes were prepared in present study, one was optimized functional multi-grain instant *idli* mix (FMIIDM) which was prepared with optimized quantity of RE, UD, SR, MPP and CR. The final proportion of FMIIDM contained Q parts RE, S parts UD, T parts SR as nutria-cereal, V parts MPP as a functional ingredient and Y g curd along with adjuncts was prepared with all ingredients together. The compositional evaluation of optimized functional multi-grain instant *idli* mix and control *idli* mixes (CM1 and CM2) was carried out by using methods reported in section 3.3.1.

Table 4.36 Proximate composition of optimized functional multi-grain instant *idli* mix (FMIIDM) and control mixes (CM1 and CM2)

Composition	CM1	CM2	FMIIM
Total moisture (% by wt.)	4.37^{c} ±0.12	5.32^{a} ±0.04	5.62^{a} ±0.12
Total carbohydrate (% by wt.)	79.22^{a} ±0.15	80.8^{b} ±0.23	65.34^{c} ±0.28
Total protein (% by wt.)	9.06^{b} ±0.25	7.21^{c} ±0.14	16.53^{a} ±0.25
Total fat (% by wt.)	4.97^{b} ±0.03	3.97^{c} ±0.03	9.03^{a} ±0.09
Total ash (% by wt.)	2.38^{c} ±0.03	2.70^{b} ±0.05	3.50^{a} ±0.17

CM_1-Control *Idli* mix-1; CM_2-Control *Idli* mix-2; Functional Multi-grain Instant *Idli* Mix (FMIIDM); CID_1-Control *Idli*- 1; CID_2-Control *Idli* -2 and Functional Multi-grain Instant *Idli* (FMID)

Mean ±SE, (n=3) Mean values with different superscripts are significantly different with each other ($p<0.05$) within a same row

CD (Moisture) = 0.29 CD (Carbohydrate) = 0.63 CD (Protein) =0.63

CD (Fat) = 0.16 CD (Ash content) = 0.29

It can be observed from Table 4.36 that CM1, CM2 and FMIIDM contained 9.06±0.25%, 7.21±0.14% and 16.53±0.25% protein content, 79.12±0.23%, 80.74±0.15%, 65.34±0.28% carbohydrate content, respectively. The fat (%) and ash (%) content of CM1, CM2 and FMIIM were 4.97±0.03, 3.97±0.03 and 9.03±0.09; and 2.38±0.03, 2.70±0.05 and 3.50±0.17, respectively. It can be clearly observed that the protein, fat and ash content of FMIIDM was significantly higher than both the control samples which could be contributed by MPP and SR, while carbohydrate was higher in CM2 (80.74±0.15%). Further, minerals like calcium, iron and magnesium are higher in *Moringa oleifera* pods (Aslam *et al.,* 2005) as well as SR (Pontieri *et al.,* 2014), and *Moringa* seeds has oil content of about 33.23 to 40.9% (Anwar *et al.,* 2005).

4.10 FTIR spectral analysis

FTIR of different *idli* mixes was carried out and is given in Fig.4.4. According to Nandiyanto *et al.,* (2019), -OH or H_2O (hydrate) functional groups absorb IR in the range of 3250 to 3650 cm^{-1}; while primary, secondary and tertiary amines absorb IR in the range 1020 to 1650 cm^{-1}; 1130 to 1650cm^{-1} and 1150 to 1210 cm^{-1}, respectively. The primary, secondary and tertiary amines are basic units for building a protein. However, proteins also experience an absorption from 1517 to 1526 cm^{-1}. Fat is reported to show absorption of IR between 2923 to 2920cm^{-1}, while carbohydrate showed an absorption from 960 to 1130cm^{-1} wavenumber. The cyanates and cyanides

absorb IR in range of 2150 to 2280 cm^{-1}. The unsaturated bonds (double and triple) of olefin compounds absorb IR at 1620 to 1670 cm^{-1}.

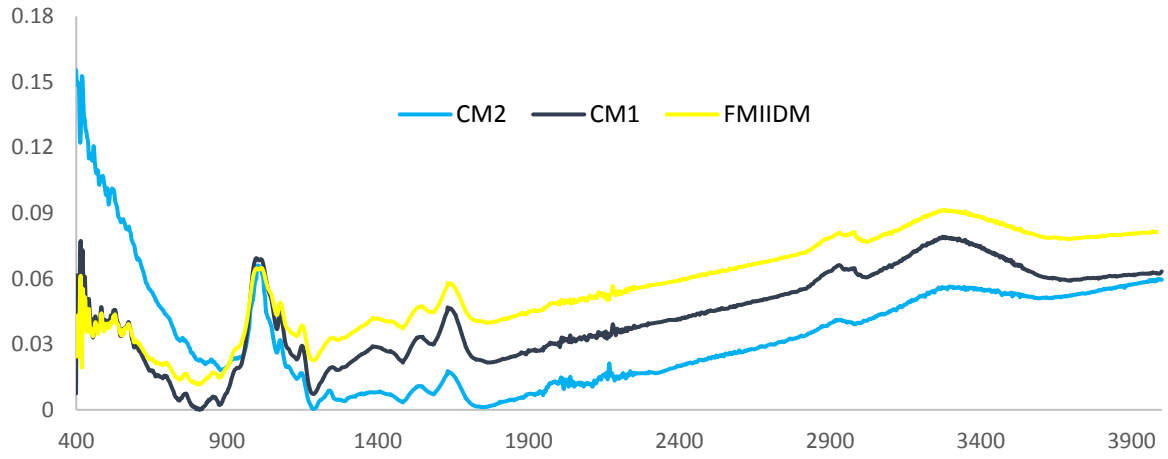


Fig 4.4 FTIR spectral analysis of *Idli* Mixes

The hydrate group represents moisture content of mixes and was observed from the peak of absorption. Fig 4.4 and Table 4.37 revealed that moisture content was high in FMIIDM followed by CM2 and CM1 found at 3286cm^{-1}. According to AOAC, Amir *et al.,* (2013) have analyzed chemical composition of wheat. The researcher found that the water molecule absorbs IR (H and OH bond) and gave peak between 1640 cm^{-1} and 3300 cm^{-1} and peak for moisture in the present study was also obtained in the range as mentioned in previous study. Olefin represent fat in mix which was high in FMIIDM>CM2>CM1 found at 1652.9cm^{-1}. Crude fat content of flour showed peak between the range of 1600 cm^{-1} and 1700cm^{-1}. Fat content of *idli* mixes was observed at 1652.9 cm^{-1} wavenumber as noted in previous study. The protein was observed high for FMIIDM>CM2>CM1 and was observed from 1552.69cm^{-1} wavenumber. Crude protein of wheat flour was obtained in range between 1550 cm^{-}1 to 1700 cm^{-1} (AOAC). It is confirmed that the peak of *idli* mix at 1552.69cm^{-1} showed crude protein of *idli* mixes. Carbohydrate absorbed IR at 999.12cm^{-1} and in the order ofCM1>CM2>FMIIDM, respectively. Rahman *et al.,* (2019) found total carbohydrates of faba using FTIR in range of 1186 to 939 cm^{-1} with the peak obtained at 1012 cm^{-1}. In present study, carbohydrate peak was obtained in between range which was also mentioned in previous study. Cyanates and cyanides were absent in control and optimized *idli* mixes, which can be determined from absence of peak in the range of 2150 to 2280 cm^{-1}. It can be

observed from Table 4.37 that wavenumber corresponding to moisture, protein and fat content of FMIIDM was significantly ($p<0.05$) higher than CM1 and CM2. Further, the wavenumber corresponding to carbohydrate content showed lowest absorbance for FMIIDM, indicating its lowest content in FMIIDM. These results are in accordance with the proximate composition of *idli* mixes as discussed in the preceding section indicating highest values of these components in FMIIDM (Table 4.36).

Table 4.37 FTIR absorption value of FMIIDM, CM1 and CM2 at their respective wave length

FTIR wavenumber (cm^{-1})	Major responsible Component	CM1	CM2	FMIIDM
3286	**Moisture content**	$0.056^{c} \pm 0.001$	$0.079^{a} \pm 0.0$	$0.089^{a} \pm 0.01$
997.19	**Carbohydrate**	$0.064^{a} \pm 0.005$	$0.069^{b} \pm 0.0$	$0.057^{c} \pm 0.02$
1552.69	**Protein**	$0.010^{c} \pm 0.001$	$0.033^{b} \pm 0.0$	$0.047^{a} \pm 0.01$
1652.9	**Fat**	$0.017^{c} \pm 0.002$	$0.045^{b} \pm 0.0$	$0.056^{a} \pm 0.01$

Mean ±SE, (n=3) Mean values with different superscripts are significantly different with each other ($p<0.05$) within a same row

CD(Moisture)=0.010 CD(carbohydrate)=0.004 CD(Protein)=0.010 CD(Fat)=0.010

4.11. Physico-chemical attributes of dry instant *idli* mixes

The physico-chemical attributes of dry instant idli mixes (Table 4.38) are discussed in this section.

Table 4.38 Physico-chemical attributes of dry instant *idli* mixes/ batter/ *idli**

Physico-chemical attributes	Sample		
	CM1	CM2	FMIIDM
L* value	$83.57^{a} \pm 0.13$	$81.87^{b} \pm 0.04$	$77.08^{c} \pm 1.05$
a* value	$0.06^{c} \pm 0.05$	$0.27^{b} \pm 0.15$	$1.8^{a} \pm 0.14$
b* value	$11.3^{c} \pm 0.02$	$14.66^{b} \pm 0.19$	$15.16^{a} \pm 0.29$
a_W	$0.33^{c} \pm 0.001$	$0.36^{b} \pm 0.0003$	$0.37^{a} \pm 0.0003$
Acidity% (Mix)	$0.27^{a} \pm 0.003$	$0.27^{a} \pm 0.003$	$0.27^{a} \pm 0.003$
pH (Batter)	$5.41^{a} \pm 0.003$	$5.41^{a} \pm 0.003$	$5.41^{a} \pm 0.003$
Acidity (% lactic acid in batter)	$0.71^{a} \pm 0.01$	$0.71^{a} \pm 0.003$	$0.71^{a} \pm 0.01$
Number of pores (*idli*)	$115.67^{b} \pm 0.67$	$121.33^{a} \pm 0.67$	$115.67^{b} \pm 0.67$

*** batter/ *idli* is written wherever application, else the analysis was conducted on dry mix**

Mean ±SE, (n=3) Mean values with different superscripts are significantly different with each other ($p<0.05$) within a same row;

CM1-Control *Idli* mix-1; CM2-Control *Idli* mix-2; FMIIDM -Functional Multi-grain Instant *Idli* Mix;

CD(L*value) = 1.72; CD(a*value) = 0.34; CD(b*value) = 0.57; CD(a_W)= 0.001;

CD(acidity of mix) =0.01; CD(pH) =0.01; CD(acidity of batter) = 0.02; CD(No. of pores) =1.64

4.11.1 Color value

4.11.1.1 L* value

L* is the measure of lightness of the sample. Its value ranges between 0 to 100 which denotes black to white color, respectively. From Table 4.38 it can be observed that L* value of all the dry mixes was significantly ($p<0.05$) different than each other. Further, L* value was significantly ($p<0.05$) lower for FMIIDM than CM1 and CM2. This could be attributed to the addition of MPP.

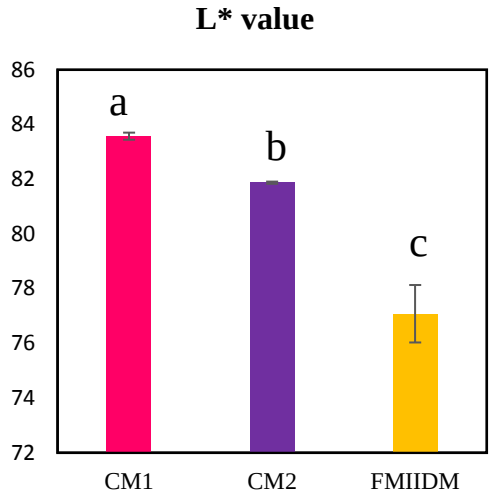


Fig 4.5 (a) L*value of control and optimized instant *idli* mix

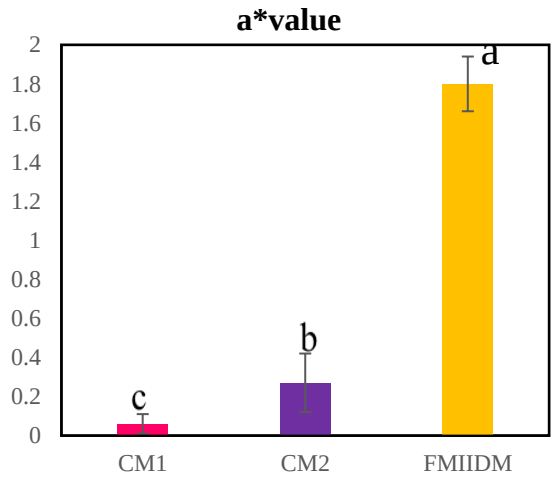


Fig 4.5 (b) a*value of control and optimized instant *idli* mix

L* value was significantly highest ($p<0.05$) for CM1 which was prepared without MPP and SR; followed by CM2 which was prepared without addition of MPP, but contained SR. It can be inferred from these observations of L* value that addition of MPP resulted in more decrease in lightness (L*) value over the addition of SR. Balasubramanian *et al.*, (2015) have reported that the addition of millet i.e., little millet into *idli* batter and its fermentation resulted in decrease of brightness i.e., L* value. This study also noted that L* value of unfermented batter and millet flour added untreated batter was ranged between 90.43 and 93.47 and >56.98 (L* value) was noted as acceptable and according to the present study, L* value of CM1, CM2 and FMIIDM were higher (i.e., 83.57, 81.87 and 77.08, respectively) than the noted L* value of previous study. Another study noted that effect of different type of dryers on colour of ready-to-reconstitute *idli*.

Padmashree *et al.,* (2014) have done drying of rice and urad dhal grits for reconstitution and they found that L* values for cabinet dried *idli* was 62. 35.

4.11.1.2 a* value

The redness of product is denoted by a* value and ranges from +60 to -60 i.e., red to green. The a* value of FMIIDM, CM1 and CM2 were significantly ($p<0.05$) different than each other and are given in Fig.4.5 (b). It can be observed from Table 4.38 the descending order of a* value of instant *idli* mixes was FMIIDM > CM2>CM1. It was noted that MPP treated i.e., FMIIDM showing highest a* value (1.8±0.14) and the slight reddish tinge was expressed by all mixes as all values were more than 0. Basuny and Marzouq (2015) reported that *Moringa* seed oil is in yellow color at 35 value and showed red unit (2±0.33) which was due to presence of natural pigments in seeds.

The red tinge in FMIIDM could be attributed to the presence of this natural pigment in MPP. Padmashree *et al.,* (2014) noted that due to browning of rice and urad dhal grits duing drying led to increase the redness value i.e., a* value on dried *idli*. Moreover, drying of fruits and vegetables increased the browning reactions (non-enzymatic) due to presence of high amount sugar and water. These kind of above mentioned non-enzymatic reactions were observed on kiwi fruit drying (Maskan, 2001). Hence, drying of grains and MPP could be the reason for increased redness (i.e., a* value) on dry mixes.

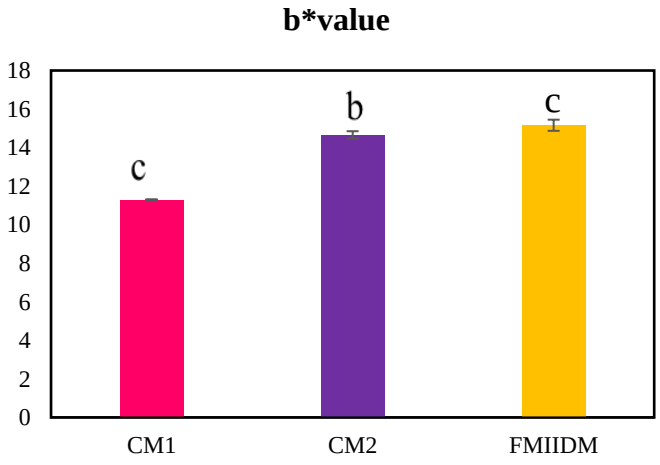


Fig 4. 5 (c) b*value on control and optimized instant *idli* mix

4.11.1.3 b* value

The b* value indicates yellowness of product and ranges from +60 (yellow) and -60 (blue). The yellowish of the instant *idli* mixes was significantly ($p<0.05$) different than each other and

was in descending order of FMIIDM > CM2>CM1.Similar to the results of a* value, MPP treated mix i.e., FMIIDM showed highest yellowish tinge than instant *idli* mix containing SR i.e. CM2, while the control containing neither MPP nor SR showed least yellowness i.e. CM1 (Table 4.38 and Fig. 4.5 c). Balasubramanian *et al.,* (2015) noted that addition of little millet flour in *idli* batter and its subsequent fermentation has led to decrease in b* value than control. But, SR (sorghum) added *idli* and MPP added *idli* mix showed high b*value (i.e., yellowness) than unfermented or control *idli* batter, which was in contrast with present study i.e., yellowness increased by replacing RE and UD with SR and/or MPP. Padmashree *et al.,* (2014) noted high b*value i.e., 6.85 in cabinet dried *idli*s than other type of dryers. Kotwaliwale *et al.,* (2007) has revealed that drying of mushrooms increased yellowness but decreased L* value and vice versa while rehydration process.

4.11.2 a_w (water activity)

Water activity (a_w) of dry mixes was in the range of 0.33 to 0.37 and it was significantly ($p<0.05$) different in all the samples (Table 4.38 and Fig. 4.6). The highest a_w was obtained for FMIIDM. This could be due to hygroscopic nature of MPP. Water activity provides an idea about stability of product over period of storage time or can noted as an intrinsic factor of food, i.e., if a_w is low then the product will have higher shelf life and vice versa. Water activity also influences chemical reactions in dried foods. Abdel and Omran (2014) noted that the a_w of instant soup mixes were in the range of 0.38±0.01 to 0.41±0.01 at 32°C. Entire microbial activity is reported to be inhibited below a_w of 0.6. Optimum a_w for bacteria, fungi and yeast was reported to be 0.9, 0.7 and 0.8, respectively (Fellows, 2000). However, even low a_w helps for oxidative and browning reactions i.e., 0.2 to 0.4 (Raitio *et al.,* 2011).

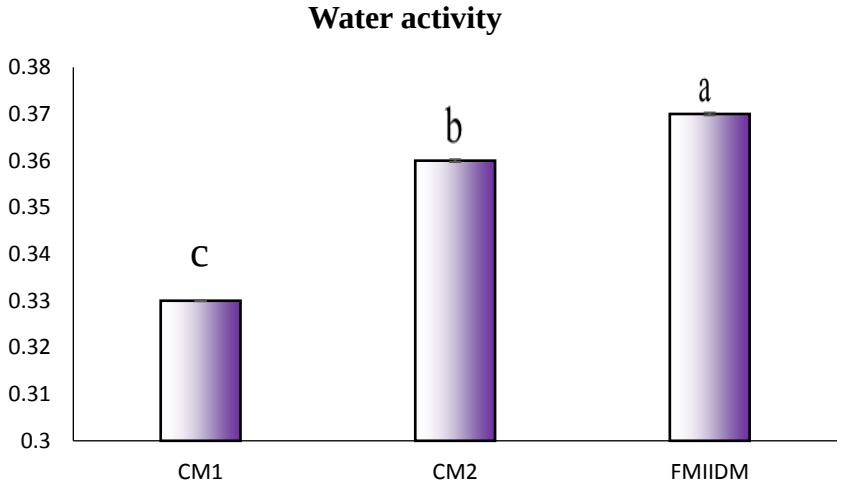


Fig 4.6. Water activity of CM1, CM2 and FMIIDM

4.11.3 Acidity and pH

Acidity of mixes (Fig 4.7 a) can be attributed to the presence of tartaric acid and malic acid. From Table 4.38, it can be noted that acidity (%) was non-significantly ($p>0.05$) different for all the mixes. This may be due to addition of equal amount acids in all mixes. Further, sour and standardized curd was used to prepare *idli* batter. The acidity (lactic acid, %) of curd was 1.39%. The acidity of batters was ~0.71 LA% (FMIIDM, CM1 and CM2) and it differed non-significantly ($p>0.05$) amongst the batters prepared from different mixes (Table 4.38) as same curd was used for preparation of batter and acidity of batter was majorly dependent on the acidity of curd. Similar results were obtained with the pH of batter prepared from all the dry mixes i.e., FMIIDM, CM1 and CM2 i.e., these differed non-significantly ($p>0.05$) from each other (fig 4.7 b). Ghosh and Chattopadhyay (2011) and Balasubramanian and Viswanathan (2006) found that acidity and pH of *idli* batter ranged from 0.44 to 0.91% and 4.2 to 5.9, respectively. In this present study, the acidity of mix was lower than the acidity of batter. This could be due to the addition of CR while preparing batter and also release of CO_2 (air pockets formation) and leavening while adding water. Further, it can be attributed to formation of carbonic acid from CO_2 and water. Balasubramanian and Viswanathan (2006) noted the increase of fermentation time caused high acidity and leavening action. Increasing of acidity in inversely related to the pH of mix/ batter.

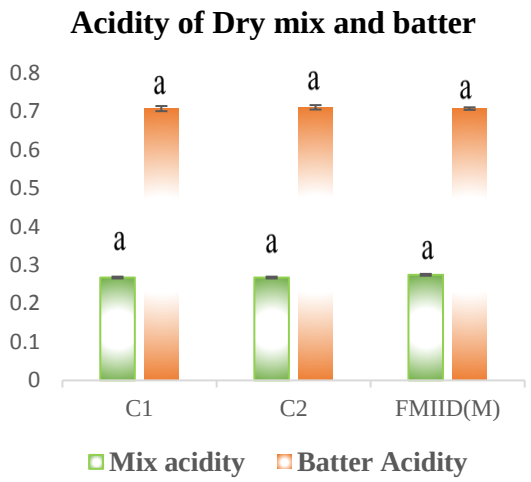


Fig 4.7(a) Acidity (%) of CM1, CM2 and FMIID(M)

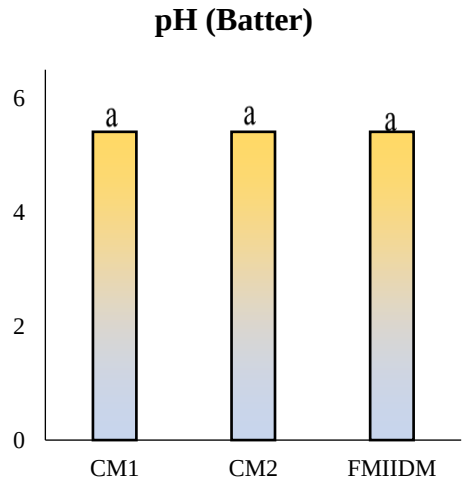


Fig 4.7(b) pH of CM1, CM2 and FMIIDM

4.11.4 Internal structure by ink print test

In ink print test, the number of observed pores in the *idli* presented per square of graph sheet are noted (Fig 4.8 (b)). If the number of pores is higher, the *idli* is softer in nature. Table 4.38

and Fig 4.8 (a) showed that the *idli* prepared from CM2 (i.e. CID2) showed the highest number of pores than that prepared from CM1 (i.e. CID1) and FMIIDM (i.e. FMIID). Chellaih *et al.*, (2016) revealed that *idli* prepared from fermented *idli* batter showed a high number of pores than *idli* prepared from fermented batter containing curry leaves. This could be due to leavening action of CO_2 microbes while fermenting batter but leavening action was low due to anti-microbial effect of curry leaves. Vanithasri and Kanchana (2013) revealed that the number pores were higher (softer) in banyard millet added *idli* than standard *idli*. Similarly, the present study also revealed that SR alone added control i.e., CID2 showed high softness (Fig 4.8 b) than FMID and CID1. On the contrary, Nazini and Shalini (2010) revealed greater number of pores obtained from standard *idli* followed by mixed *idli* and pearl millet added *idli*. In present study, MPP and SR added *idli* showed lowest softness but the pores numbers fells in the range of market samples. This could be attributed to addition of fiber rich MPP.

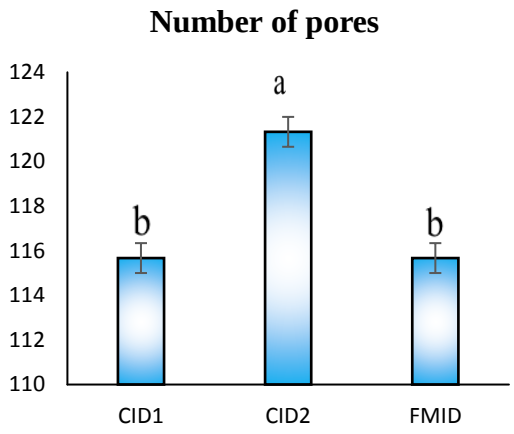


Fig 4.8 (a) Number of pores CID1, CID2 and FMID

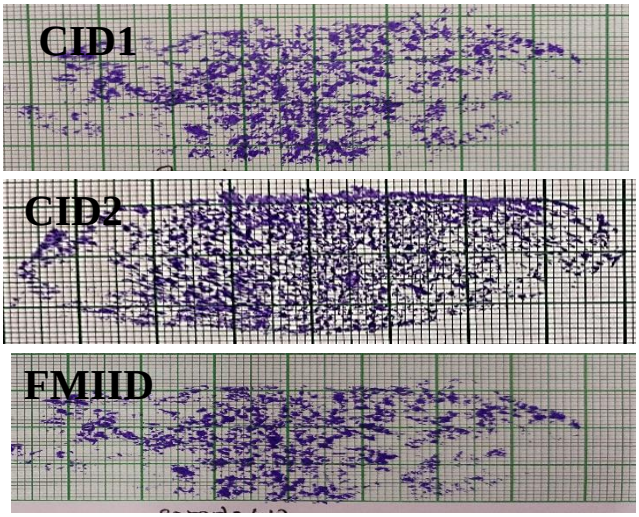


Fig 4.8 (b) Ink print test

4.12 Bio-functional attributes of dry instant *idli* mixes

4.12.1 Antioxidant activity

4.12.1.1 DPPH (2,2- Diphenyl-1-picrylhydrazyl) antioxidant assay

The major principle applied in antioxidant activity by DPPH assay is based on free-radical quenching capacity of antioxidant present in the food article, which results in reduction of purple colour of DPPH free radical and is measured by taking absorption at 517nm.

The antioxidant reaction of free radicals leads to the formation of yellow 2,2-diphenyl1picrylhydrazyl. This causes the color to disappear, which leads to a decrease in the absorption level. Thus, DPPH antioxidant activity is monitored by decreasing the absorbance.

Table 4.39 shows the antioxidant activity of instant dry mixes (CM1, CM2 and FMIIDM) in terms of DPPH.

Table 4.39 Bio-functional attributes of dry mixes FMIIDM, CM1 and CM2

Sample	**CM1**	**CM2**	**FMIIDM**
Antioxidant activity			
DPPH Scavenging activity (%)	50.76 ^{c} ±0.15	70.03 ^{b} ±0.15	76.60 ^{a} ±0.00
FRAP (µgTrolox eq./ml)	357.22 ^{c} ±0.33	529.55 ^{b} ±0.29	1319.72 ^{a} ±0.33
ABTS (µgTrolox eq./ml)	370.10 ^{c} ±0.28	437.61 ^{b} ±0.56	487.34 ^{a} ±0.28
Other biofunctional attributes			
Total phenolics (µg GAE/ml)	169.25 ^{c} ±2.98	223.53 ^{b} ±0.26	394.63^{a} ±1.88
Total flavonoids (µg QE/ml)	31.54 ^{c} ±0.10	37.90 ^{b} ±0.27	76.08^{a} ±0.10
Total carotenoids (µg/g)	4.92 ^{c} ±0.03	8.20 ^{b} ±0.02	26.59^{a} ±0.00
Vitamin C (mg/100 g)	22.03 ^{b} ±2.75	16.52^{c} ±0.00	85.64 ^{a} ±2.76
Calcium (mg/g)	24.63^{c} ±2.90	50.98 ^{b} ±0.34	116.57 ^{a} ±0.67
Iron (mg/g)	2.18 ^{c} ±0.01	2.30^{b}±0.02	3.45 ^{a} ±0.05

Mean ±SE, (n=3) Mean values with different superscripts are significantly different with each other ($p<0.05$) within a same row.

CD (DPPH) =0.35 CD (FRAP) = 0.90 CD (ABTS) =1.11
CD (total phenolics) =5.78 CD (total flavonoids) =0.5 CD (total carotenoids) =0.07

The results revealed that DPPH activity of all the dry mixes was significantly ($p<0.05$) different than each other with FMIIDM showing the highest DPPH scavenging activity (76.60±0.00%) followed by CM2 (70.03±0.15) and CM1 (50.76±0.15).

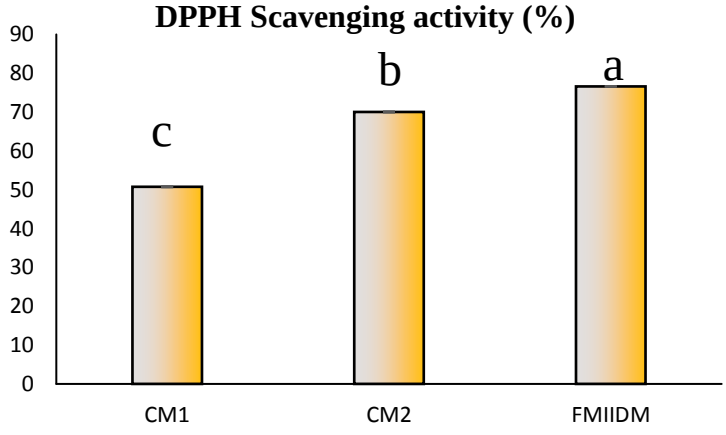


Fig 4.9 DPPH scavenging activity of CM1, CM2 and FMIIDM

High percentage of DPPH scavenging activity in FMIIDM may be due to the high reducing power of anti-oxidants of MPP as well as SR than RE based *idli* mix (CM1). This further reveal

that MPP with SR resulted in higher DPPH activity in comparison to the sample containing SR alone. Fig.4.9 illustrates the DPPH scavenging activity of CM1, CM2 and FMIIDM.

4.12.1.2 FRAP assay

The principle of working of this method is reduction of Fe^{3+} TPTZ complex (colourless) to Fe^{2+} tripyridyltriazine (blue coloured) formed by the action of electron providing antioxidant at low pH. The colour changes were measured at 593nm. Lapsongphon *et al.,* (2013) found that the heat treated moringa pulp and seed showed ferric reducing capacity of 2.87 and 2.05 mg ferrous eq./g respectively and which could be due to the presence of carotenoids and flavonoids. Shen *et al.,* (2018) found that white sorghum showed ferric reducing activity 82.85% at 2.02 mgVceq./g. This could be the reason for significantly ($p<0.05$) high FRAP antioxidant value of FMIIDM (1319.72 µg Trolox eq/ml) followed by CM2 (529.55 µg Trolox eq/ml) than and (357.22 µg Trolox eq/ml). Fig 4.10 and Table 4.39 showed the value of FRAP antioxidant assay of *idli* mixes.

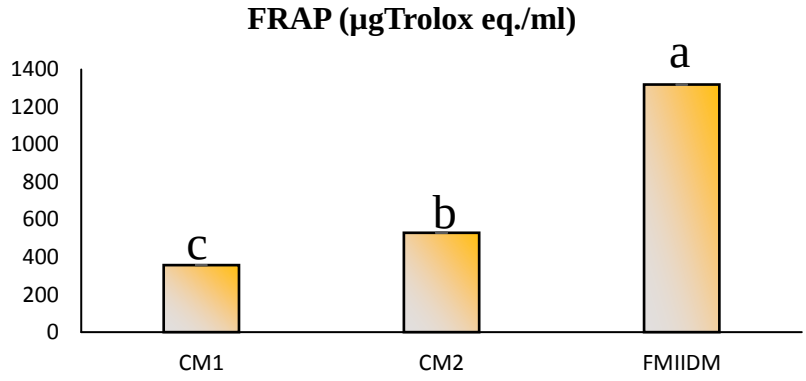


Fig 4.10 FRAP activity of CM1, CM2 and FMIIDM

Luqman *et al.,* (2011) reported that the extract of the *Moringa oleifera* leaves and pods both showed high anti-oxidant activities during *in vitro* experiments than *in vivo* with ethanolic and aqueous extract, respectively. These workers also found that reducing power of *Moringa* fruit ethanolic extract in terms of DPPH and FRAP was significantly ($p<0.0001$) higher among extract of leaf in water and ethanol as well as fruit aqueous extract. The values obtained in present study are in accordance with the literature.

4.12.1.3 ABTS assay

It can be observed from Table 4.39 and Fig 4.11 that the ABTS activity of all the dry mixes was significantly ($p<0.05$) different than each other. However, the highest ABTS assay was obtained in FMIIDM (487.34±0.28 µg Trolox eq/ml) followed by CM2 (437.61±0.56 µg Trolox

eq/ml) and CM1 (370.10±0.28 µg Trolox eq/ml). Mohamad and Manan (2015) found phenolics and flavonoids content in seeds of *Moringa oleifera* and reported a value of 10.179 mg gallic acid eq. /g and 2.9 mg Quercetin eq/g respectively. The highest ABTS activity of FMIIDM could be attributed to the presence of phenolics and flavonoids in MPP. Dhawi *et al.,* (2020) found that *Moringa oleifera* seed flour added yoghurt had highest total phenolic content (31.61mg GAE/g) with anti-oxidant activity (89.32%). They also revealed that total phenolics and anti-oxidant activity of *Moringa oleifera* seed flour was higher than fenugreek seed flour.

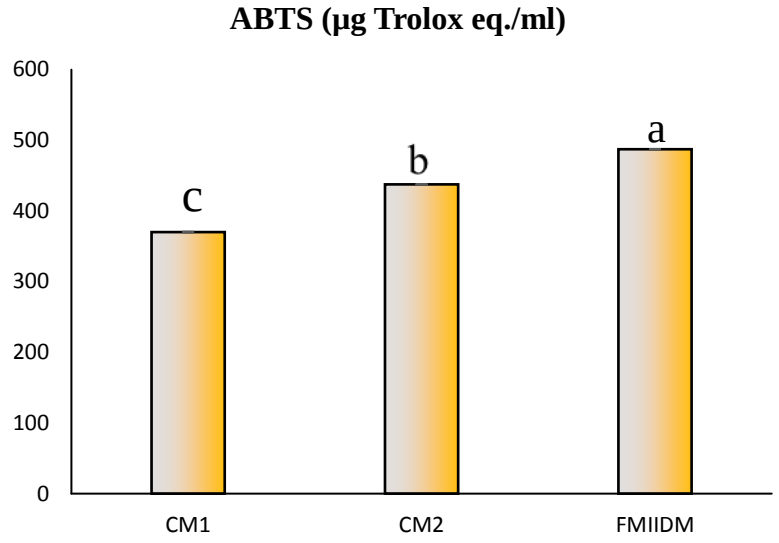


Fig 4.11 ABTS scavenging activity of CM1, CM2 and FMIIDM

4.12.2 Total phenolics content

The total phenolic content of *idli* mixes were determined by prussian blue method of Graham (1992). The total phenolics were expressed in microgram gallic acid equivalent per ml of extract. Table 4.39 and Fig 4.12 revealed that phenolic content of all the three different dry mixes had significantly ($p<0.05$) different total phenolic content. FMIIDM had highest total phenolics (394.63±1.88µg GAE/ml) followed by CM1 (223.53±0.26 µg GAE/ml) and CM2 (169.25±2.98 µg GAE/ml). The significantly ($p<0.05$) higher phenolics of FMIIDM could be attributed to the addition of MPP and SR. According to Lapsongphon *et al.*, (2013), the total phenolics present in heat treated pulp and seed of *Moringa oleifera* was 1.19±0.02 and 0.91±0.01 mg gallic acid eq/g (100mg powder/ml extract), respectively. Massry *et al.,* (2013) revealed that the phenolic compounds (quercetin, caffeic acid and kaemperol) were enriched in pods and seeds of *Moringa*

oleifera. The increase in total phenolics in FMIIDM than CM1 and CM2 could be attribute to the replacement of RE and UD with SR and MPP.

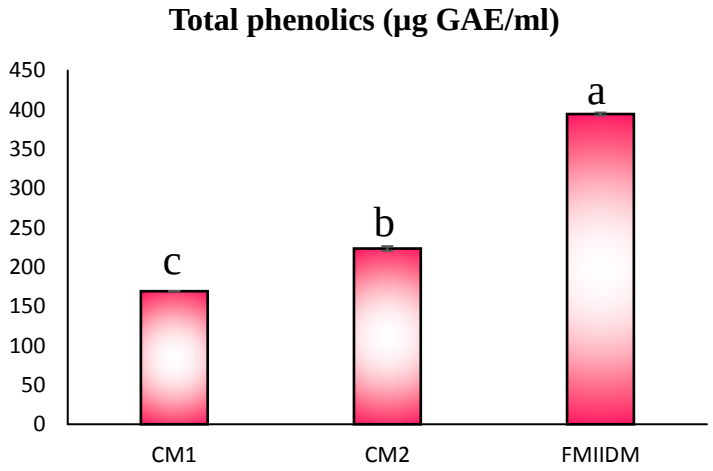


Fig 4.12 Total phenolics of CM1, CM2 and FMIIDM

4.12.3 Total flavonoids content

Total flavonoids content was analyzed by aluminum chloride method. Free and bound total flavonoids in white sorghum was 11.18±1.65 mg RE/100g and 0.54±0.06, respectively (Shen *et al.,* 2018) and *Moringa* seeds contained total flavonoids of around 2.9 ±0.002 mg QE/g on dry matter. Table 4.39 shows total flavonoids of *idli* mixes and reveals a significantly ($p<0.05$) different content of total flavonoids in all the *idli* mixes. It is clear that MPP and SR added *idli* mixes i.e. FMIIDM (76.08±0.10 µg QE/ml) showed highest flavonoid content followed by CM2 (37.90±0.27µg QE/ml) which contained only SR. The flavonoid content of CM1 was lowest, i.e. 31.54±0.10 µg QE/ml, which could be attributed to the absence of MPP and SR in its preparation. Since white SR was used in the present study which lacks flavonoids and is tannin free. Therefore, the flavonoid content of CM2 was close to that of CM1. Xu *et al.,* (2019) proved the positive correlation among the anti-oxidant and anti-microbial activity with total flavonoids content of *Moringa oleifera* parts i.e., leaves and seeds and they revealed that *Moringa oleifera* parts are highly suitable for the development of dietary supplements. The higher flavonoid content in FMIIDM could be attributed to the presence of higher contents of flavonoids as reported in preceding study.

4.12.4 Total carotenoids content

Total carotenoids of different mixes is shown in Table 4.39. The total carotenoids content of all the mixes differed significantly than each other, i.e., 26.59±0.00µg/g in FMIIDM, 8.20±0.02

µg/g in CM2 and 4.92±0.03 µg/g in CM1. According to Yooying *et al.,* (2019), the beta carotenoid content in boiled and steamed pods (pulp and seed) was 210 to 243 µg/100g and 152 to 220 µg/100g. CM1 showed lowest carotenoids. This could be due to the presence of hardly any

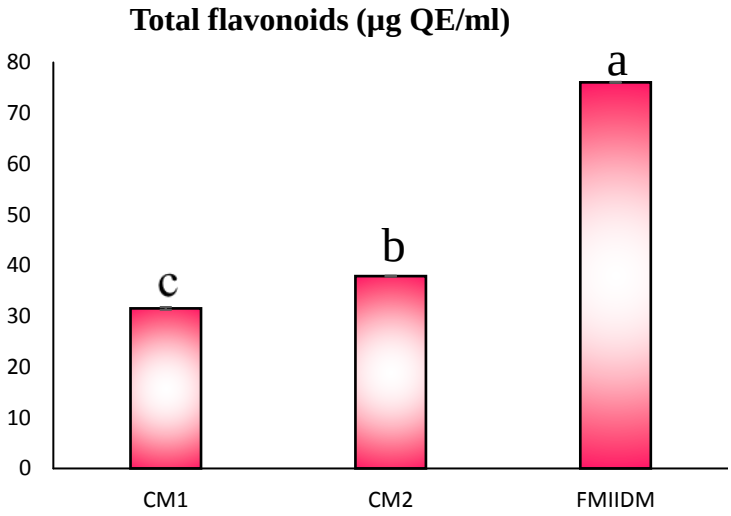


Fig 4.13 Total flavonoids of CM1, CM2 and FMIIDM

carotenoid in dehulled black gram cotyledons (0.042 mg/g) than other fraction of black gram (Girish *et al.,* 2012). Similarly, brown rice also has less amount of beta carotene and lutein (100ng/g); and zeaxanthin 30ng/g. Saini *et al.,* (2014) revealed presence of carotenoids in vegetative and reproductive parts of *Moringa oleifera* (i.e., leaves, flowers and fruits). They found 6 predominant carotenoids i.e., all-E-luteoxanthin, 13-Z-lutein, all-E-lutein, all-E-zeaxanthin, 15-Z-beta-carotene and all-E-beta-carotene and reported that leaves had high amount of carotenoids than fruits. The results are in accordance with the literature cited.

4.12.5 Vitamin C

Vitamin C was evaluated in *idli* mixes by titration method. The possible sources of vitamin C in the mixes could be MPP, fenugreek and fermentation. The vitamin C of all samples was significantly ($p<0.05$) different than each other. FMIIDM showed highest vitamin C content followed by CM1 (22.03±2.75mg/100g) and CM2 (16.52±0.00mg/100g) having significantly high vitamin C than CM1 and CM2 and the source of vitamin C is. Ahmed *et al.,* (2016) found that L-ascorbic acid in *Moringa oleifera* pods are 3.96 to 8.27 mg/100g. RE, SR and UD have no vitamin C. Though CM1 and CM2 having known amount of vitamin C i.e., was due to fermentation of

grains. Masih *et al.,* (2019) noted that vitamin C in fresh fruits of *Moringa* was 120 mg/100g. Therefore, the concentrated form of MPP could be the reason for high Vitamin C in FMIIDM.

4.12.6 Iron content

Iron content of different mixes revealed in Table 4.39. MPP and SR treated FMIIDM (3.45±0.05mg/100g) and SR alone treated CM2 (2.30±0.02) showed significantly ($p<0.05$) high in iron content than CM1 (2.18±0.01mg/100g). Anwar *et al.,* 2005 have found iron in *Moringa oleifera* pods were 155.2 to 435.9 mg/kg. SR, UD and RE iron content 5.54 to 7.65mg/100g (Afify *et al.,* 2011), 4 to 7.55mg/100g (Modgil *et al.,* 2019), 6.9 to 22.3mg/kg (Maganti *et al.,* 2020) respectively. Dhawi *et al.,* (2020) incorporated 0.1% and 0.2% of *Moringa* seed flour in to yoghurt and which led to increase content of minerals i.e., Ca, P, K and Fe in the end product. Similarly, the higher iron content of *idli* mix (FMIIDM) over CM1 and CM2.

4.12.7 Calcium content

Calcium content of *Moringa* pods was reported to be 1292 to 1837mg/kg (Afify *et al.,* 2011), while that of urad dal ranged from 147.79 to 155.62mg/100g (Modgil *et al.,* 2019) and sorghum ranged from 9.59 mg to 67.16mg/100g (Tasie and Gebreyes, 2020). Because of natural calcium present in ingredient used in samples, the FMIIDM showed highest calcium content 116.57±0.67mg/100g as observed from Table 4.39 followed by CM2 (50.98±0.34mg/100g) and CM1 (24.63±2.90mg/100g).

4.13 Rheology parameters of batters of FMIIDM, CM1 and CM2

4.13.1 Flow curve of *Idli* batter from FMIIDM, CM1 and CM2

The data of shear rate (τ) and shear stress (γ) for FMIIDM, CM1 and CM2 are plotted in Fig 4.14. Power law equation (Oswald-de Waele model, $\tau=k\gamma^n$) was used to calculate the flow behavior index (n) and consistency index (k) of batters which were treated with MPP and SR (FMIIDM), SR alone (CM2) and untreated (CM1). The power law model was fitted on data of flow curve for FMIIDM, CM1 and CM2 and flow behavior index (n) of FMIIDM, CM1 and CM2 was 0.30±0.01, 0.25±0.01 and 0.26±0.01, respectively. The flow behavior index of all batters showed non-significant ($p<0.05$) difference between them. Based on flow behavior index noted in Table 4.40, it can be revealed that flow behavior ($n<1$) of batter prepared from FMIIDM, CM1 and CM2 showed non-Newtonian fluid behavior i.e. pseudoplastic behaviour. From Fig 4.15, it can be observed that the apparent viscosity decreased as shear rate increased, which expressed shear thinning effect of batter in all mixes. Consistency index (k) and behavior index (n) are the

indices for characterizing shear thinning or pseudoplastic fluids and consistency index gives idea about viscosity of batter. Further, increase in consistency index also increased the viscosity of product. Consistency index (k) was significantly ($p<0.05$) higher for CM2 (69.75±0.28) than CM1 and FMIIDM. This may be due to the free sugars liberated in batter from sorghum during fermentation. FMIIDM and CM1 showed non-significant ($p>0.05$) difference in consistency index. It was justified from Table 4.37 (proximate) that the amount carbohydrate in CM1 and FMIIDM was lower than CM2.

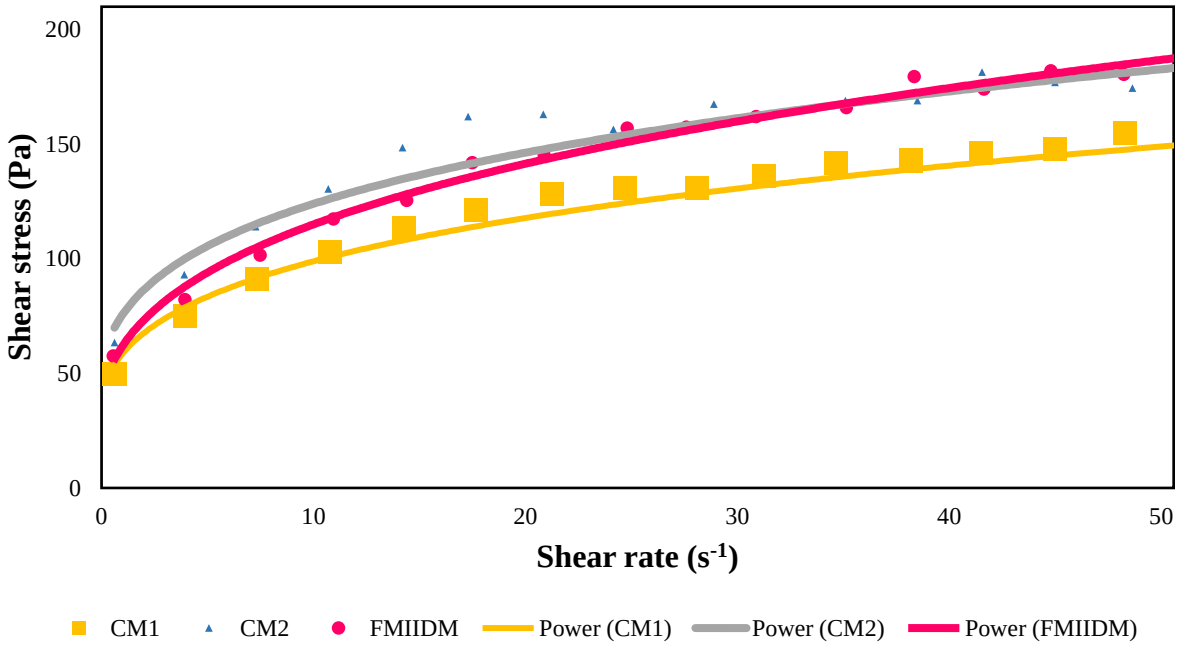


Fig 4.14 Flow curve of batter from control and optimized mix

Apparent viscosity of all mixes decreased on increasing shear rate over period of time (0 to 100s^{-1}). The decreasing apparent viscosity over a period of time led to increase in the flow of product (thicker to thinner). Similarly, the increase in shear rate decreased apparent viscosity (Fig 4.15), which revealed that increasing shear rate of batter led to thin batter from thick batter. This could be due to escape of CO_2 produced by action of acid and $NaHCO_3$ in the presence of moisture during formation of batter. It can be concluded from these observations that for obtaining a fluffy or spongy *idli*, over mixing or blending of batter while preparation should be avoided.

4.13.2 Frequency sweep

Amplitude sweep was done to find LVR (linear viscoelastic region) where G' and G" were constant. This constant value is also called as plateau value. From Fig 4.16 (a), 4.16 (b) and 4.16 (c), it can be observed that G'=G" where strain was 63.3%, 25.77% and 40% known as gel point

of batter prepared from FMIIDM, CM1 and CM2, respectively and this revealed the viscoelastic solid nature of batter. From amplitude sweep, 0.1% of strain was fixed for further analysis i.e., frequency sweep.

Table 4.40 Consistency and Flow behavior index of CM1, CM2 and FMIIDM

Sample	Consistency index (k)	Flow behavior index (n)
CM1	53.90 [a] ±0.19	0.26 [a] ±0.15 (n<1)
CM2	69.75 [b] ±0.28	0.25 [a] ±0.20 (n<1)
FMIIDM	55.98 [a] ±0.20	0.30 [a] ±0.08 (n<1)

Mean ±SE, (n=3) Mean values with different superscripts are significantly different with each other ($p<0.05$) within a same column.

CD (consistency index) = 2.18 CD (Flow behavior index) = 0.001

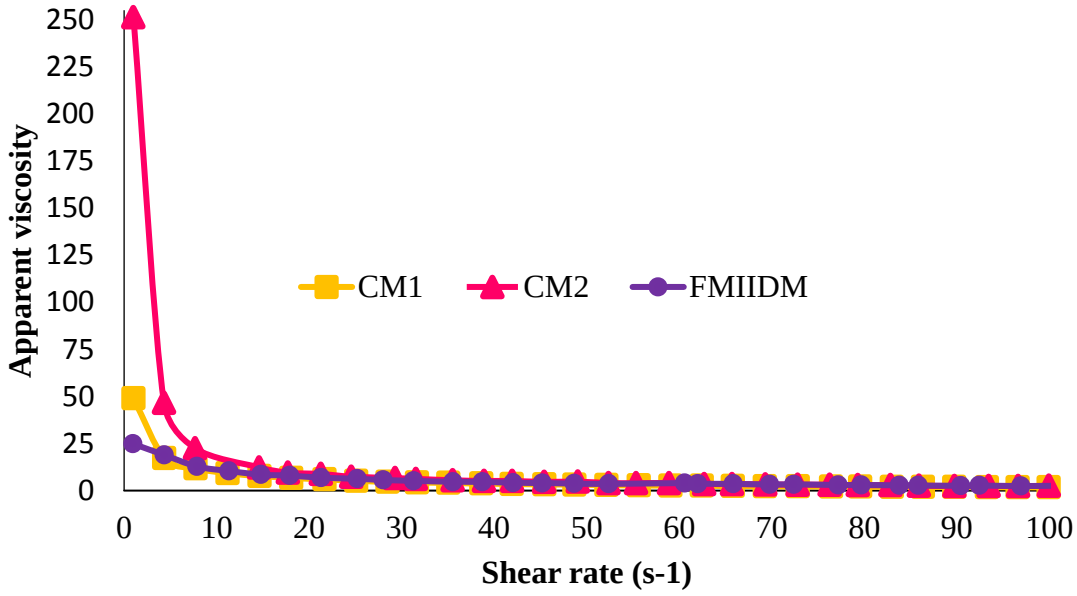


Fig 4.15 Apparent viscosity with respect to shear rate

Fig 4.17 showed that the dynamic mechanical behavior of batter prepared from FMIIDM, CM1 and CM2 at the angular frequency between 0-100 s^{-1}. Over this range, storage/ elastic modulus G' was higher than loss/ plastic modulus G", which confirmed that the batter prepared from FMIIDM, CM1 and CM2 exhibited the viscoelastic behavior with solid like characteristics. Therefore, the batter of FMIIDM, CM1 and CM2 have a tendency to behave mostly like solid. The

graphs of G' and G" denotes the strong gel like attribute of the batters. All batters showed elastic behavior dominantly (G'>G") over the frequency of 0 to 100 s^{-1}. Matos *et al.,* (2014); and Matos and Rosell (2014) found that with or without addition of source of external protein into rice-based flour, G'of the batter or dough increased than G" in dynamic spectra.

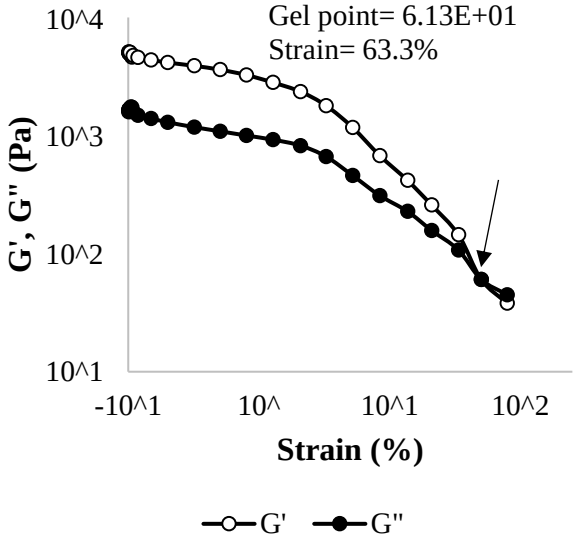


Fig 4.16 (a) Amplitude sweep of FMIIDM

From Fig 4.17, it can be concluded that batter prepared from MPP and SR containing FMIIDM showed high viscoelastic behavior than the batters prepared from CM1 and CM2. Hence, the batter prepared from FMIIDM is expected to show high firmness to the steamed end product i.e. *idli* (FMID) than CID1 and CID2. This can also be inferred from Table 4.41 that *idli* from FMIIDM showed hardness significantly ($p<0.05$) higher than CM1 and CM2 and CM2 batter showed lowest G'modulus than CM1 and FMIIDM, which showed that the firmness or hardness of *idli* will be lower than *idli* from other batters (CM1 and FMIIDM). This is also justified from number of pores on *idli* of CM2 (Table 4.38).

4.13.3 Thermo-rheological properties by temperature sweep

The storage and loss modulus values showed subsequent changes which were monitored and noted by raising temperature of batter prepared from FMIIDM, CM1 and CM2 from 20 to 100°C a given in Fig 4.18. From figure 4.18, it is noted that storage and loss modulus showed similar trends for all batters. The temperature at which the sudden increase in elastic or storage modulus takes place marks the initiation of gelatinization and Matos *et al.,* (2014) determined the gelatinization temperature of batter using temperature sweep test data. Batter prepared from CM1, CM2 and FMIIDM showed that initiation of gelatinization took place at temperature of 63.4°C,

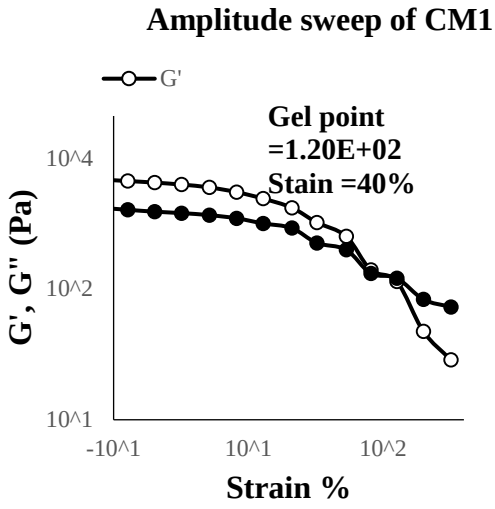


Fig 4.16 (b) Amplitude sweep of CM1

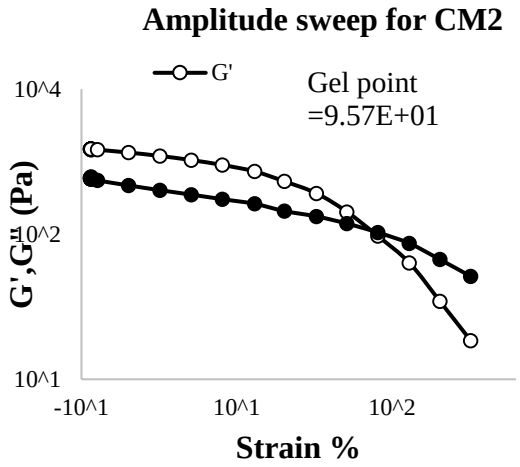


Fig 4.16 (c) Amplitude sweep of CM2

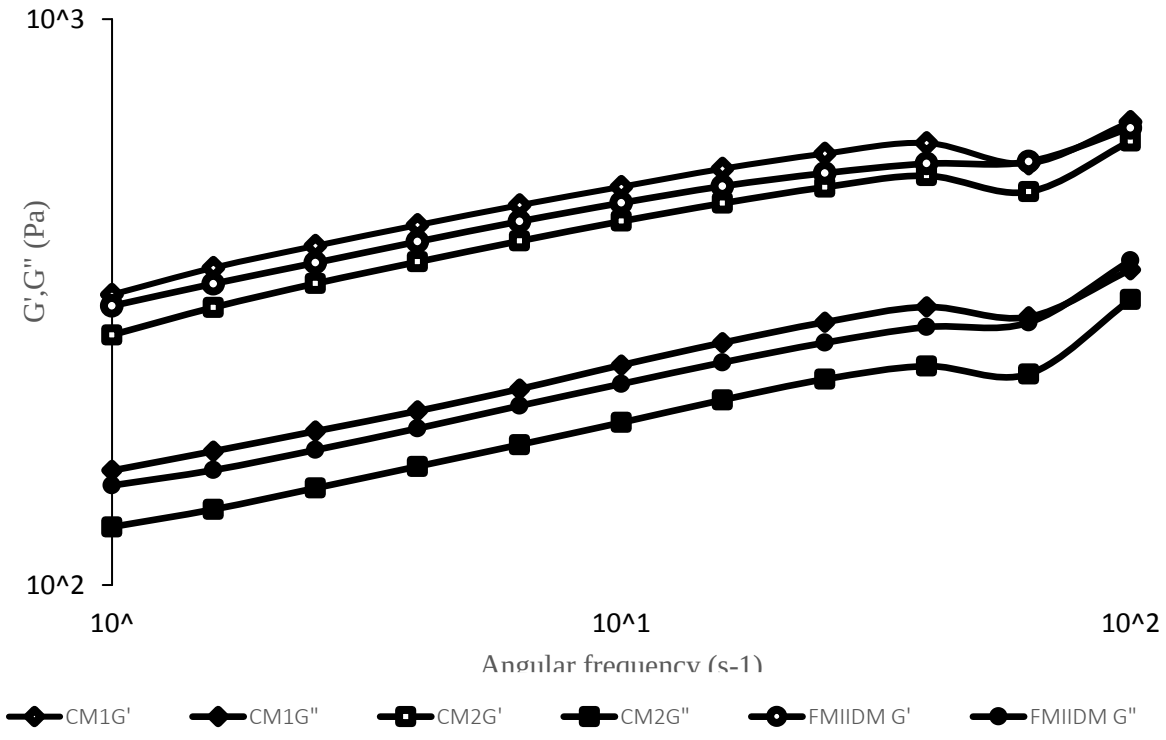


Fig 4.17 Frequency sweep of FMIIDM

61.8°C and 65°C, respectively. As this specific gelatinization temperature is reached, this phase contains an environment rich in amylose due to hydrogen bonding. However, the combination of water and heat caused amylose to be destroyed by water absorption and leached amylose from the

starch, which caused the gel to gelatinize or soften by forming a 3D network (Upadhyay and Mehra, 2017). The gelatinization temperature of batter prepared from FMIIDM (65°C) was highest followed by CM1 (63.8°C) and CM2 (61.8°C).

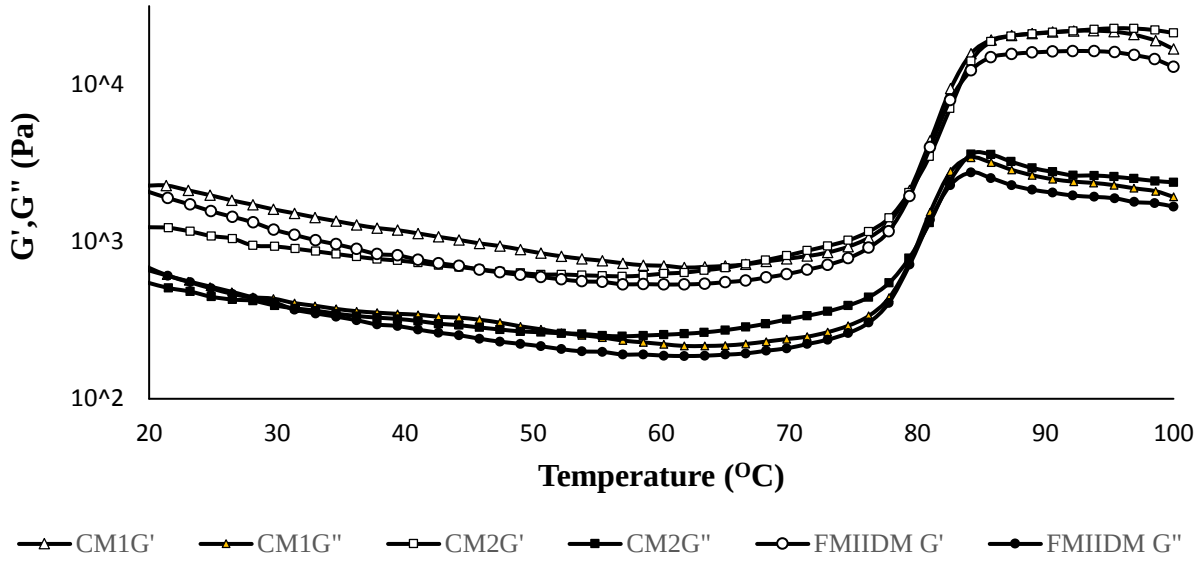


Fig 4.18 Thermo-rheological properties of all mixes

The increase of gelatinization temperature in batter prepared from FMIIDM could be due to addition of protein of UD and MPP in FMID as protein has the tendency to increase gelatinization temperature due to formation of complex with starch molecules on the surface of starch molecules, which subsequently hinders amylose from leaching (Sumnu *et al.,* 1998). High G' value and increased elastic modulus explains the well cross-linked network structure of starch (Upadhyay and Mehra, 2017; Sumnu *et al.,* 1998). During starch gelatinization, the amylose molecules swells and soften the matrix, thus changing the batter from liquid to solid form. Subsequently, increasing temperature caused decrease in G' (elastic modulus) of FMIIDM at 93.8°C, CM1 at 96.9^{O}C and CM2 at 95.37^{O}C which indicated break down of gel network or melting of crystal amylopectin leading to increase of viscosity or softening of gel network.

4.14 Texture profile analysis of FMID, CID1 and CID2

4..14.1 Hardness

Hardness of *idli* (Fig 4.19 a) is maximum force required at first compression which is simulation of force expressed in first bite at maximum force. It is expressed in Newtons. Texture profile parameters of CID1, CID2 and FMID are shown in Table 4.41 and which clearly demonstrate that hardness was significantly ($p<0.05$) higher in FMIIDM (6.83±0.04N) than CID1

(6.16±0.14N). Though, it showed non-significant ($p>0.05$) difference with CID2, which was prepared using RE, UD and SR. This indicated that hardness was similar for both MPP and SR treated sample. SR alone treated sample i.e., CID2 (5.82±0.42N) showed significantly ($p<0.05$) low hardness than FMIIDM but showed non-significant ($p>0.05$) difference with CM1 hardness. However, the hardness of all *idli*es fell in the range of hardness of market *idli* samples (from Table 4.4).

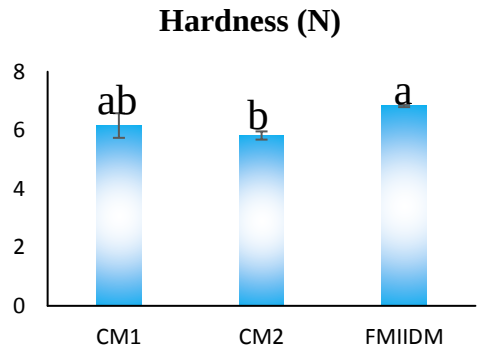


Fig 4.19 (a) Hardness of CM1, CM2 and FMID

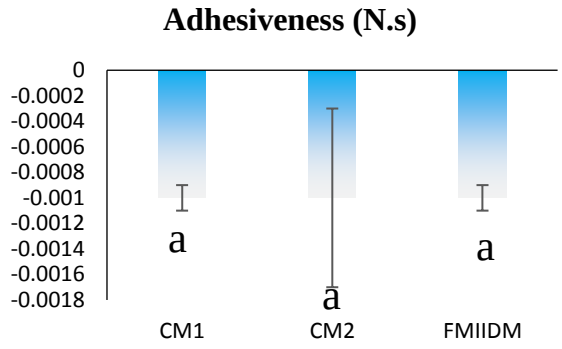


Fig 4.19 (b) Adhesiveness of CM1, CM2 and FMID

Rice to dhal ratio and fermentation have been reported to affect the hardness of *idli* (Kumari *et al.*, 2020) and the authors also noted that parboiled rice and fermentation based *idli* showed lower hardness in the range of 5.43N to 8.98N. In this study, due to the fiber content of MPP in FMID showed hardness than other two samples i.e. CM1 and CM2. Notably, fermentation of whole batter is likely to cause perfect leavening than fermenting as whole grains

4.14.2 Adhesiveness

Adhesiveness is work needed to overcome the attractive forces between food and palate of mouth. It is expressed in N. s. Higher adhesiveness is correlated with the higher stickiness of *idli* (Pawar *et al.*, 2018; Shoba and Joshi, 2018). So, adhesiveness for *idli* should be in the minimum range. Adhesiveness of CID1, CID2 and FMID is shown in Table 4.41 and Fig 4.19 b, which revealed that the adhesiveness of all *idli* samples showed non-significant ($p>0.05$) effect with the values close to -0.001N.s. This value fells in the range of adhesiveness of market samples of *idli* i.e., -0.5 to 0 N.s (from Table 4.4). Maheswari and Shetty, (2013) found that adhesiveness of *Moringa oleifera* leaves added *idli* ranged from -0.94 to -1.08 N.s, which was lower than control samples. FMID showed very minimum adhesiveness than previously studied *idli* i.e., *Moringa* leaf added *idli*.

Table 4.41 Texture profile analysis of FMID, CID1 and CID2

Sample	CID1	CID2	FMID
Hardness (N)	6.16 ^{ab} ±0.14	5.82 ^{b} ±0.42	6.83 ^{a} ±0.04
Adhesiveness (N.s)	-0.001 ^{a} ±0.007	-0.001 ^{a}±0.001	-0.001 ^{a} ±0.001
Springiness (mm)	1.36 ^{a} ±0.09	1.59 ^{a}±0.13	1.34 ^{a} ±0.02
Cohesiveness	0.13 ^{a} ±0.01	0.15 ^{a} ±0.01	0.15 ^{a} ±0.01
Chewiness (J)	1.11 ^{a} ±0.12	1.45 ^{a} ±0.13	1.35 ^{a} ±0.04
Resilience	0.48 ^{a} ±0.02	0.53 ^{a} ±0.05	0.50 ^{a} ±0.02

Mean ±SE, (n=3) Mean values with different superscripts are significantly different with each other ($p<0.05$) within a same row

CD (Hardness) =0.73 CD (Adhesiveness) =0.002 CD (Springiness) =0.25
CD (Cohesiveness) =0.02 CD (Chewiness) =0.54 CD (Resilience) =0.09

4.14.3 Springiness

Softness of the *idli* is expressed by springiness and it depends on the quantity of black gram dhal with respect to rice used for preparation of *idli* (Shoba and Joshi, 2018; Maheswari and Shetty, 2013). It can be defined as the regaining of height of *idli* during the resting period after first compression (Kumari *et al.*, 2020). Fig 4.19 C and Table 4.41 showed that the springiness of FMID

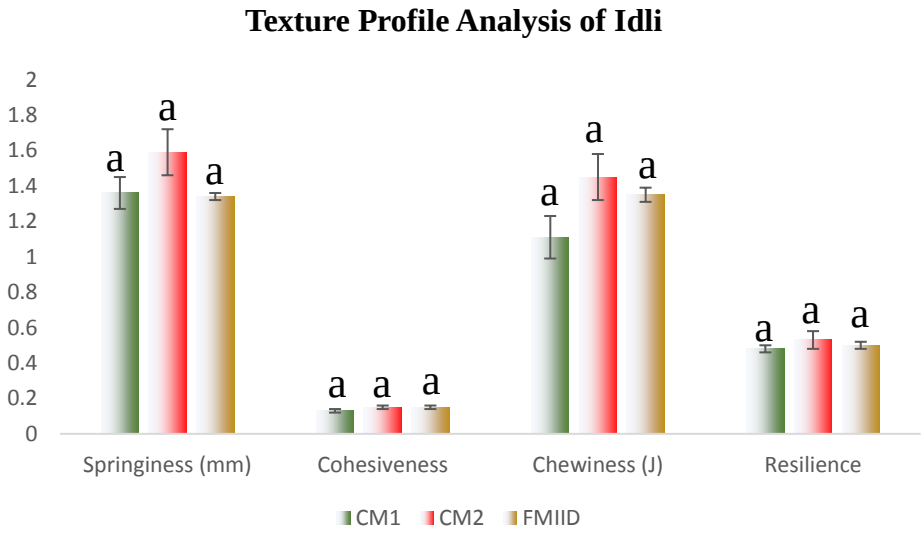


Fig 4.19 (c) Springiness, Cohesiveness, Chewiness and Resilience of CM1, CM2 and FMID

(1.36±0.02mm), CID2 (1.59±0.02mm) and CID1 (1.36±0.02mm) were non-significantly ($p>0.05$) different than each other. It was interesting to note that all the *idli* samples i.e., control and test showed higher values of springiness (Table 4.41) than the market *idli*es (0.79mm to 0.88mm, Table

4.4). As per Maheswari and Shetty (2013), the highest springiness (i.e., 0.78 mm) was obtained with lowest concentration of *Moringa* leaf i.e., 5%. It is clear that *idli* prepared from FMIIDM, CM1 and CM2 obtained higher springiness than previously prepared *idli*.

4.14.4 Cohesiveness

Cohesiveness denotes the internal strength of food matrix (Kumari *et al.,* 2020), so with increase in cohesiveness, less deformation is expected in the product. Fig 4.19 (C) and From Table 4.41, it is cleared that the cohesiveness of FMID (0.15), CID2 (0.13) and CID (0.15) were similar and non-significantly ($p>0.05$) different. Cohesiveness of these samples were lesser than the market sample as given in Table 4.4 (0.78 to 0.88). Maheswari and Shetty (2013) noted that the cohesiveness ranged between 0.61 to 0.78. Durgadevi and Shetty (2012) have noted that increased urad dhal level with constant rice level increased cohesiveness from 0.526 to 0.912. So, it is important that addition of urad dhal should be higher for obtaining low cohesiveness of *idli*, This could be due to the gas holding capacity of black gram dhal components (i.e. globulin and arabinogalactan). In present study, adjuncts were added for texture improvement which might have helped to decrease cohesion and showed low cohesiveness than found in previous study.

4.14.5 Chewiness

Chewiness is the energy released during mastication of food, which is required to change solid form of food to eatable form (Kumari *et al.,* 2020). Therefore, it is expressed as Joules (J) or milli joule (mJ) and lower chewiness representing softer the *idli*. According to the Table 4.41, it can be observed that energy expelled while *idli*s prepared from various mixes were chewed was non-significant ($p>0.05$) different between samples and the values for different mixes were FMID (1.35±0.04 J), CID2 (1.45±0.13 J) and CID1 (1.11±0.12 J). Durgadevi and Shetty (2012); Maheswari and Shetty (2013) noted that the chewiness of *idli* was 1389.17 mJ to 2557.13 mJ and 765.8 mJ to 1543.17 mJ, respectively. FMID, CID1 and CID2 showed minimum chewiness which proven that the *idli*s prepared from all the mixes were softer.

4.14.6 Resilience

Resilience refers to regaining of shape of compressed product after releasing the compression during eating. The lower resilience of product represents that the product is firm (Maheswari and Shetty, 2013), while higher the resilience softer the product. Fig 4.19 (C) and From Table 4.41, the different *idli*s were non-significantly ($p>0.05$) different than each other i.e., FMID (0.50±0.02), CID2 (0.53±0.05) and CID1 (0.48±0.02). Resilience of treated sample and

control also satisfied the range of market samples resilience i.e., 0.29 to 0.73. Resilence of *Moringa* leaves added *idli* ranged from 0.35 to 0.47 (Maheswari and Shetty, 2013). Durgadevi and Shetty (2012) found that the resilience 0.285 to 0.654. Resilience of FMID, CID1 and CID2 fell in the range as mentioned in above studies.

4.15 Sensory attributes of FMID, CID1 and CID2

The sensory attributes of FMID, CID1 and CID2 studied were colour, dryness interior, porosity, firmness, springiness, fermented aroma, acidic taste and overall acceptability.

4.15.1 Color

From Fig 4.20 and Table 4.42, the colour scores of FMID was 4.2±2.37, while lowest value was obtained for other *idli*es (CM1 and CM2) and significantly ($p<0.05$) differed. But, CID1 and CID2 were non-significantly ($p>0.05$) different than each other. Based on the score values, it can be inferred that the slightly darker color of FMID could be due to addition of MPP than SR in its instant mix. On the other hand, SR added *idli* (CID2) showed non-significant ($p>0.05$) score with CID1. This drastic change in color could be attributed to the presence of carotenoids, phenolics, flavonoids in *Moringa* as well as drying of *Moringa* pulp which leads to colour change because of non-enzymatic browning due to presence of high protein in *Moringa* seeds (Liang *et al.,* 2019) and sugars in *Moringa* pods.

4.15.2 Dryness interior

Dryness of interior was significantly ($p<0.05$) high in FMID than CM1 and CM2 due to hygroscopic property of MPP. CID1 obtained 2nd highest dryness interior score i.e., 5±0.13 and lowest dryness interior score was obtained for 3.66±0.35 (CID2). Though these values were optimal for *idli* and were in the range of market *idli* dryness (Table 4.42) and score was in range of slightly wet to moderately wet.

4.15.3 Porosity

Porosity of *idli* could be attributed to $NaHCO_3$, leavening acids (tartaric acid and malic acid), steam and sour dahi. Chemically, leavening acids induce $NaHCO_3$ to release CO_2 after adding water as well as due to heat treatment. From Table 4.42 and Figure 4.20, it can be observed that the porosity of all *idli*es (FMID-6.84±0.25, CID2-6.71±0.5 and CID2-6.60±0.25) showed non-significant ($p>0.05$) scores which could be due to the equal amount of all leavening ingredients. The porosity of all *idli*es also obtained optimal scores which fell in range with the market *idli* samples (i.e. optimally porous to definitely porous).

Table 4.42 Organoleptic properties scores of FMID, CID1 and CID2

Attributes	CID1	CID2	FMID
Color	9.86 [a] ±0.29	8.94 [b] ±0.46	4.2 [c] ±2.37
Dryness interior	3.66 [c] ±0.35	5 [b] ±0.13	5.62 [a] ±0.19
Porosity	6.60 [a] ±0.25	6.71 [a] ±0.5	6.84 [a] ±0.25
Firmness	4.32 [a] ±0.18	4.28 [a] ±0.34	4.48 [a] ±0.53
Springiness	5.38 [a] ±0.55	5.3 [a] ±0.7	5.3 [a] ±0.49
Fermented aroma	5.4 [a] ±0.46	5.68 [a] ±0.31	5.84 [a] ±0.31
Acidic taste	5.34 [a] ±0.32	5.14 [a] ±0.09	5.84 [a] ±0.16
Overall acceptability	8.56 [a] ±0.22	8.36 [a] ±0.34	8.84 [a] ±0.34

Mean ±SE, (n=3) Mean values with different superscripts are significantly different with each other ($p<0.05$) within a same row

CD (Color) = 0.58 CD (Dryness interior) = 0.32 CD (Porosity) = 0.46
CD (Firmness) = 0.49 CD (Springiness)=0.76 CD (Fermented aroma) = 0.48
CD (Acidic taste) = 0.34 CD (Overall acceptability) = 0.40

4.15.4 Firmness

Firmness of all *idli*es were in the range of optimum (definitely soft to moderately firm). The scores for firmness for all *idli*es were 4.48±0.53 for FMID, 4.28±0.34 for CID2 and 4.32±0.18 for CID2 (Figure 4.20 and Table 4.42). Though texture profile analysis showed hardness higher for FMID than CID1 and CID2 but sensory scores were non-significantly ($p>0.05$) different than each other. Perdon *et al.,* (1999) stated that cooling also increases firmness due to retrogradation of starch retrogradation.

4.15.5 Springiness

Springiness was due to CO_2 holding capacity of ingredients in *idli*. Generally, springiness of naturally fermented *idli* is due to components present in black gram dhal i.e., arabinogalactan and globulin. Viscoelastic properties is obtained in gluten free ingredients by using starch with some polysaccharide and water for stabilizing network for gas holding (Demirkesen *et al.*, 2010). Springiness of non-fermented gluten free rice based steamed cake was obtained successfully using hydrocolloids, which was obtained by improving viscoelastic behavior of batter as well as gas holding capacity during baking (Itthivadhanapong *et al.*, 2016). Similarly, while processing of flaked rice, starch was converted to resistant starch (Kumar *et al.*, 2018). This resistant starch played crucial role in gluten free baked items by forming viscoelastic properties and increasing springiness (Witzak et al, 2016). Hence, based on preliminary trials gum Acacia was used along with flaked rice powder for improving spongy texture or gas holding capacity. From Table 4.42,

and Figure 4.20, it can be observed that all the *idli*es scored non-significant ($p<0.05$) scores for springiness expressing same level of springiness for *idli*es (i.e. FMID- 5.3±0.49, CID1-5.38±0.7 and CID2-5.38±0.55). Springiness was in the range i.e. moderately springy to nearly springy.

4.15.6 Fermented aroma and Acidic taste

Fermented aroma and acidic taste in *idli*es could be attributed to the presence of sour dahi and acid with yeast extract. All *idli*es were in the range of moderate to definite and scored for fermentation aroma and acidic taste FMID-5.84±0.31, CID1- 5.68±0.31, CID2-5.4±0.46 and FMID-5.84±0.16, CID1-5.14±0.09, CID2-5.34±0.32 respectively. These scores showed non-significant ($p>0.05$) from each other (Table 4.42 and Figure 4.20).

4.15.7 Over all acceptability

Overall acceptability of *idli* like products mainly depends on soft and spongy texture, taste, and color of product. All these parameters together contribute to overall acceptability of *idli*. The overall acceptability also scored in the range 'liked moderately to liked extremely' i.e. the scores

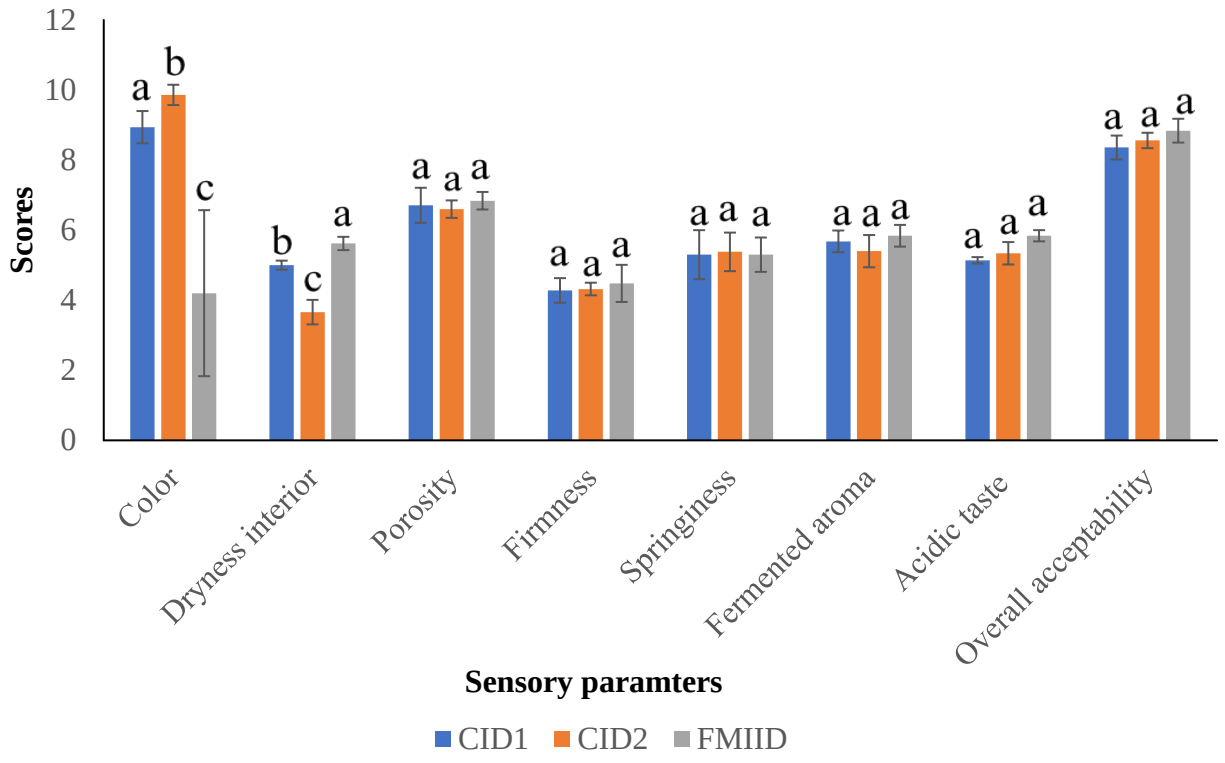


Fig 4.20 Sensory scores of CID1, CID2 and FMID

were 8.84±0.34 for FMID, 8.36±0.34 for CID1 and 8.56±0.34 for CID2 and showed non-significant (p>0.05) difference, though FMID scored highest. Thus, it can be concluded that treated *idli* (FMID) was equally accepted like control *idli*es (CID1 and CID2) and highly accepted organoleptically than market *idli*es and which scored 5.07 to 7.77. (Table 4.42).

4.16 Microbial analysis of FMIIDM, CM1 and CM2

4.16.1 Total plate count

Total plate count is shown in Table 4.43 and it can be observed that all mixes showed non-significant (p>0.05) log cfu/g. The TPC for *idli* batter was noted to be 9.20±0.18 after 12 hour (Regubalan and Anandhanarayan, 2018). *Idli* mixes showed lower TPC (17 hours fermented as whole grains) than naturally fermented batter.

4.16.2 Yeast and mold count

Yeast and mold count can be observed from Table 4.43. All the mixes showed non-significant (p>0.05) log cfu/g i.e. for FMIIDM, CM1 and CM2. The yeast and mold for fermented batter was noted to be 7.94±0.02 after 12 hours (Regubalan and Anandhanarayan, 2018). *Idli* mixes showed lower TPC than naturally fermented batter.

Table 4.43 Total plate count and yeast & mold count of *Idli* mixes

Sample	Total Plate Count [log (cfu/gm)]	Yeast and mold
CM1	3.393 [a] ±0.003	1.98 [a] ±0.02
CM2	3.394 [a] ±0.003	1.97 [a] ±0.03
FMIIDM	3.392 [a] ±0.003	1.97±0.03

Mean ±SE, (n=3) Mean values with different superscripts are significantly different with each other (p<0.05) within a same column.

CD (TPC) = 0.002 CD (yeast and mold) = 0.08

4.16.3 Coliforms

The presence of coliforms in product represents the unhygienic processing method. In product prepared in the present study, coliforms count was nil in 10 grams expressing that *idli* mixes were prepared in hygienic environment.

CHAPTER 5

Summary and Conclusion

5.0 SUMMARY AND CONCLUSION

Health concern of consumers as well as changing lifestyle lead to researches for development of healthy and instant diet which also requires lots of understanding of the subject as well as innovative approaches. Further, incorporation of a functional ingredient into food plays a crucial role in addressing the issues pertaining to malnutrition and poor health. Nutri-cereals and some vegetables like *Moringa oleifera* are source of plethora of bio-active compounds like phenolics, flavonoids, total carotenoids, vitamin C and calcium which provides bio-functionality to the end product. Studies have proven that sorghum and pods of *Moringa oleifera* possess anti-diabetic, anti-carcinogenic, hypocholestremic, anti-ulcer, anti-inflammatory, anti-ageing properties, etc. In present study, the functional instant *idli* was developed. During the research work, initially the market samples were evaluated for descriptive sensorial appeal, textural profile analysis and ink print test for establishing range for these parameters which was used later during optimization of functional instant *idli* mix. The raw ingredients used for optimized were RE, UD, SR, MPP and CR and optimization was carried out based on sensorial and textural profile parameters along with ink print test using 2^5 (full factorial) experiment of CRD. The optimized combination along with control *idli* mixes were prepared and analyzed for physico-chemical, bio-functional, sensorial, rheological and microbiological parameters. The results obtained were analyzed by one way ANOVA and the research work is summarized hereunder.

5.1 Descriptive sensory profiling, Texture profile analysis and ink print test of market sample

5.1.1 The ranges for the sensory parameters like colour, dryness interior, porosity, firmness, springiness, fermented aroma, acidic taste and overall acceptability; textural parameters like hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience and ink print test (number of pores) were obtained to be 2.50 to 7.20, 4.23 to 6.50, 4.60 to 7.00, 2.83 to 7.20, 3.47 to 7.03, 1.00 to 8.37, 1.37 to 7.90, 5.07 to 7.77; ~2.5 to ~8, ~ -0.5 to 0, 0.787 to 0.884, 0.777 to 0.961, 1.706 to 6.016, 0.294 to 0.73; and 78 to 145 respectively and these were used during optimization of functional multi-grains Instant *Idli* mix

5.2 Optimization of ingredients for preparation of functional multi-grain *Idli* mix

5.2.1 The functional multi-grain instant *Idli* mix (FMIIDM) was prepared using 32 different combinations of RE, UD, SR, CR and MPP as per the 2^5 factorial experiment. The ratios of raw ingredients were optimized based on sensory parameters like colour, porosity, firmness, stickiness, springiness, fermented aroma, acidic taste and overall acceptability), textural parameters

(hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience) and ink print test (number of pores) as per the range established in 5.1.1

5.2.2 The combination of ingredients i.e. RE- Q parts, UD-S parts, SR- T parts, MPP- V parts and CR- Y g were optimized based on sensorial attributes and textural profile analysis as well as ink print test.

5.2.3 For TPA and ink print parameters of *idli* prepared from optimized mix, the hardness (3.310±0.13 N), adhesiveness (-0.016±0.01 N.s), springiness (0.666±0.01 m), cohesiveness (0.384±0.02), chewiness (0.852±0.07 J), resilience (0.202±0.01) and no. of pores (111±0.58) were obtained within the range established from market sample.

5.2.4 The sensory scores in terms of color (6.357±0.48), dryness interior (4.200±0.61), porosity (5.671±0.82), firmness (2.771±0.70), stickiness (1.129±0.76), springiness (6.243±0.61), fermented aroma (6.186±1.39), acidic taste (3.700±0.96) and overall acceptability (9.386±0.27) for optimized combination fell in the range established from market sample.

5.3 Proximate composition of optimized functional multi-grain instant *Idli* mix (FMIIM) and control mixes (CM1 and CM2)

Proximate composition of FMIIM was determined in terms of moisture (5.62±0.12%), total carbohydrate (65.34±0.28%), total protein (16.53 ±0.25%), total fat (9.03±0.09%) and total ash (3.50 ±0.17%), while proximate composition of CM1 and CM2 was also determined as moisture, carbohydrate, protein, fat and ash with respective values being 4.37±0.12% & 5.32 ±0.04%; 79.22±0.15% & 80.8±0.23%; 9.06±0.25% & 7.21 ±0.14%; 4.97±0.03% & 3.97 ±0.03%; 2.38±0.03% & 2.70 ±0.05%. Among the proximate composition, protein, fat and ash were significantly ($p<0.05$) higher in FMIIDM than control mixes (CM1 and CM2).

5.4 FTIR analysis of optimized functional multi-grain instant *Idli* mix (FMIIM) and control mixes (CM1 and CM2)

FTIR results based on the absorbance at specific wavenumber also revealed that the protein, fat and ash were significantly ($p<0.05$) high in FMIIDM than control mixes (CM1 and CM2).

5.5 Physico-chemical attributes of optimized functional multi-grain instant *Idli* mix (FMIIM) and control mixes (CM1 and CM2)

FMIIDM, CM1 and CM2 were found to have L* values of 77.08±1.05, 83.57±0.13 and 81.87±0.04, respectively. FMIIDM, CM1 and CM2 were found to have a* values of 1.8±0.14, 0.06±0.05 and 0.27±0.15, respectively. FMIIDM, CM1 and CM2 were found to have b* values of

15.16±0.29, 11.3±0.02 and 14.66±0.19, respectively. L* value was observed to be significantly ($p<0.05$) lower in FMIIDM, while a* and b* value was significantly ($p<0.05$) higher in FMIIDM. FMIIDM, CM1 and CM2 were found to have water activity of 0.37±0.0003, 0.33±0.001 and 0.36±0.0003, respectively. Water activity of FMIIDM was significantly ($p<0.05$) higher than CM1 and CM2. Acidity of all mixes non-significantly ($p>0.05$) differed from each other i.e. (0.27±0.003). Similarly, pH and acidity of all batters was non-significantly ($p>0.05$) different than each other i.e. (5.41±0.003 and 0.71, respectively). FMIIDM, CM1 and CM2 were found to have number of pores 115.67 ±0.67, 115.67±0.67 and 121.33±0.67, respectively and were significantly ($p<0.05$) higher in CM2 than CM1 and FMIIDM.

5.6 Bio-functional attributes optimized functional multi-grain instant *Idli* mix (FMIIM) and control mixes (CM1 and CM2)

Anti-oxidant assays like ABTS, FRAP and DPPH were significantly ($p<0.05$) high in FMIIDM than CM1 and CM2. Amongst two different control mixes, CM2 showed significantly ($p<0.05$) high antioxidant potential than CM1. Total phenolics, flavonoids, carotenoids, vitamin C, minerals like iron and calcium were significantly high ($p<0.05$) in FMIIDM than CM1 and CM2.

5.7 Rheological parameters of batters prepared from optimized functional multi-grain instant *Idli* mix (FMIIM) and control mixes (CM1 and CM2)

Batter from FMIIDM, CM1 and CM2 showed consistency index (k) of 55.98±0.20, 53.90±0.19 and 69.75±0.28, respectively and flow behavior index (n) of 0.30±0.08, 0.26±0.15 and 0.25±0.20, respectively which showed $n<1$ indicating the non-Newtonian behavior of batter. All these indexes revealed significant ($p<0.05$) difference between batters. Apparent viscosity of batters from all mixes decreased with increasing shear rate (s^{-1}), depicting shear thinning effect of batters. Frequency sweep of all batters showed gel point (G'=G") at 63.3%, 25.77% and 40% for FMIIDM, CM1 and CM2, respectively and all batters showed G' and G" parrel to each other with respect frequency indicating strong viscoelastic solid behavior of batters.

5.8 Thermo-rheological properties by temperature sweep

Thermo-rheological properties showed that starting temperature for gelatinization was 63.4°C, 61.8°C and 65°C for CM1, CM2 and FMIIDM, respectively observed by slight increase in storage modulus (G'). End of the gelatinization of temperature was also obtained for FMIIDM at 93.8°C, CM1 at 96.9°C and CM2 at 95.37°C.

5.9 Texture profile analysis of FMID, CID1 and CID2

Texture profile parameters like hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience were analyzed for FMIID, CID1 and CID2. All parameters showed non-

significant ($p>0.05$) difference except hardness. However, all parameters attained the range as established using market *idli* parameters.

5.10 Sensory attributes of FMIID, CID1 and CID2

Sensory attributes (descriptive score card) of *idli* prepared from FMIID, CID1 and CID2 showed that all parameters like colour, porosity, firmness, stickiness, fermented aroma, acidic taste and overall acceptability were within the range established from market *idli* samples and more acceptable than market samples. FMIID, CID1 and CID2 showed non-significant ($p>0.05$) difference in all parameters except firmness which showed significant ($p<0.05$) difference.

5.11 Microbial analysis of FMIIDM, CM1 and CM2

The standard plate count and yeast and mold of FMIIDM, CM1 and CM2 were within the normal level as observed from several cited literature. Coliforms showed nil content for 10 g of sample indicating that mix was prepared in hygienic condition.

Conclusion

The optimized functional *idli* mix contained higher protein and fat contents and showed superior bio-functional properties than control mixes without having a detrimental effect on the sensorial and textural attributes of the *idli* samples. This indicates that the developed product holds the potential to be accepted by masses and can address issues related to malnutrition due to presence of higher amount of iron, calcium and carotenoid content, etc over control. However, the health attributes in terms of bio-availability of the components are required to be studied through *in-vivo* analysis of mix and cooked *idli*, besides the storage study of developed product for drawing concrete and wholesome inference.

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ANNEXURES

Annexure 1: ANOVA for studying effect of different ingredients (individually and combination) on Texture profile attributes and ink print test for optimization of ingredients for preparation of functional multi- grain instant *idli* mix

Source	Hardness	Adhesiveness	Springiness	Cohesiveness	Chewiness	Resilience	IPT
RE	9.878**	3.159E^{-5NS}	.069*	.026^{NS}	.089^{NS}	.898*	742.594**
UD	12.499**	.000^{NS}	.011*	.015^{NS}	2.615^{NS}	.062^{NS}	481.510**
SR	.976*	7.498E$^{-5\ NS}$	.009*	.338*	8.077*	.005^{NS}	645.844**
MPP	2.115*	.000^{NS}	.006*	1.067E$^{-5\ NS}$	.026^{NS}	.373^{NS}	219.010**
CR	59.743**	.000^{NS}	.123**	1.779**	63.567**	3.811**	2233.010**
RE * UD	1.713*	.000^{NS}	4.817E^{-5NS}	.033^{NS}	.011^{NS}	.227^{NS}	1033.594**
RE * SR	1.037*	6.002E$^{-5\ NS}$	.023*	.002^{NS}	.016^{NS}	.075^{NS}	3.010^{NS}
RE * MPP	.829^{NS}	1.256E$^{-7\ NS}$	.001^{NS}	.050^{NS}	.765^{NS}	.249^{NS}	666.760**
RE * CR	3.444**	4.536E$^{-5\ NS}$	.000^{NS}	.030^{NS}	1.504^{NS}	.948*	.010^{NS}
UD * SR	6.520**	.000^{NS}	.011*	.038^{NS}	.002^{NS}	.842*	311.760**
UD * MPP	7.557**	.000^{NS}	.002^{NS}	.000^{NS}	.956^{NS}	.525*	882.094**
UD * CR	9.836**	.000^{NS}	.006*	.007^{NS}	2.132^{NS}	.047^{NS}	3.760^{NS}
SR * MPP	6.771**	.000^{NS}	.001^{NS}	.090^{NS}	5.243*	.393^{NS}	656.260**
SR * CR	16.126**	.001 *	.029**	.207*	8.061*	.015^{NS}	55.510**
MPP * CR	2.348*	.001 *	.000^{NS}	.004^{NS}	.352^{NS}	.318^{NS}	625.260**
RE * UD * SR	6.887**	.001**	.010*	.025^{NS}	1.932^{NS}	1.163*	753.760**
RE * UD * MPP	2.984**	7.151E$^{-5\ NS}$	.001^{NS}	.151*	5.810*	.344^{NS}	1592.510**
RE * UD * CR	3.207**	.000^{NS}	.002^{NS}	.057^{NS}	.002^{NS}	.273^{NS}	1254.260**
RE * SR * MPP	5.742**	1.366E$^{-5\ NS}$	.017**	.039^{NS}	1.888^{NS}	1.887**	276.760**
RE * SR * CR	.438^{NS}	1.869E$^{-6\ NS}$	.031**	.007^{NS}	.239^{NS}	.067^{NS}	4226.760**
RE * MPP * CR	.223^{NS}	.000^{NS}	3.038E$^{-5\ NS}$	.063^{NS}	.923^{NS}	.271^{NS}	201.260**
UD * SR * MPP	2.757*	6.981E$^{-\ 5NS}$	.001^{NS}	.003^{NS}	.186^{NS}	.008^{NS}	4774.260**
UD * SR * CR	.636^{NS}	3.631E-5$^{\ NS}$	.017**	.032^{NS}	.152^{NS}	.968*	6550.510**
UD * MPP * CR	1.140*	3.754E-5$^{\ NS}$	.010*	.010^{NS}	.043^{NS}	.451*	86.260**
SR * MPP * CR	.432^{NS}	7.756E-6$^{\ NS}$	1.350E$^{-5\ NS}$	.137^{NS}	2.757^{NS}	.339^{NS}	348.844**
RE * UD * SR * MPP	4.553**	.001*	.012*	.002^{NS}	1.244^{NS}	.310^{NS}	304.594**
RE * UD * SR * CR	.131^{NS}	.001*	.003^{NS}	.039^{NS}	1.058^{NS}	1.236*	207.094**
RE * UD * MPP * CR	3.411**	3.510E$^{\ -5\ NS}$	.026**	.070^{NS}	5.747 *	.375^{NS}	27.094**
RE * SR * MPP * CR	.624^{NS}	9.256E$^{-7\ NS}$	.000^{NS}	.011^{NS}	.096^{NS}	1.894**	396.094**
UD * SR * MPP * CR	.811^{NS}	1.366E$^{-5\ NS}$	.001^{NS}	.012^{NS}	.014^{NS}	.003^{NS}	3116.760**
RE * UD * SR * MPP * CR	.065^{NS}	.000^{NS}	.003^{NS}	7.042E$^{-6\ NS}$	.595^{NS}	.356^{NS}	319.010**

FMID: Functional Multi-grain *Idli*

Annexure 2: ANOVA for studying effect of different ingredients (individually and combination) on different sensory attributes studied for optimization of functional multi- grain instant *idli* mix

Source	Color	Dryness interior	Porosity	Firmness	Springiness	Fermented aroma	Acidity	Overall acceptability
RE	43.402**	.838^{NS}	6.013^{NS}	38.778*	12.970^{NS}	10.372^{NS}	19.802^{NS}	5.722^{NS}
UD	32.864*	4.658^{NS}	6.013^{NS}	.464^{NS}	7.107^{NS}	12.540^{NS}	4.458^{NS}	16.394*
SR	8.254^{NS}	1.100^{NS}	.090^{NS}	.272^{NS}	1.561^{NS}	.483^{NS}	.219^{NS}	.011^{NS}
MPP	79.206**	.225^{NS}	.116^{NS}	.206^{NS}	2.341^{NS}	3.206^{NS}	.394^{NS}	18.515*
CD	79.922**	20.825*	8.216^{NS}	55.402*	3.279^{NS}	8.409^{NS}	.521^{NS}	35.521**
RE * UD	18.862*	11.205^{NS}	33.868*	2.200^{NS}	1.186^{NS}	2.972^{NS}	.580^{NS}	30.459*
RE * SR	.004^{NS}	3.525^{NS}	3.475^{NS}	9.202^{NS}	.000^{NS}	3.018^{NS}	.086^{NS}	18.978*
RE * MPP	.560^{NS}	.766^{NS}	.055^{NS}	.046^{NS}	.354^{NS}	.411^{NS}	2.835^{NS}	.018^{NS}
RE * CR	20.040*	18.229*	1.045^{NS}	28.714*	8.064^{NS}	.009^{NS}	5.344^{NS}	.046^{NS}
UD * SR	34.414*	4.950^{NS}	3.575^{NS}	11.703^{NS}	.323^{NS}	8.643^{NS}	.540^{NS}	4.686^{NS}
UD * MPP	5.531^{NS}	2.465^{NS}	4.891^{NS}	.002^{NS}	14.658^{NS}	3.303^{NS}	6.379^{NS}	1.321^{NS}
UD * CR	15.540*	.135^{NS}	2.858^{NS}	11.340^{NS}	.145^{NS}	13.504^{NS}	16.72^{NS}	1.511^{NS}
SR * MPP	.161^{NS}	.550^{NS}	.200^{NS}	.150^{NS}	11.295^{NS}	.150^{NS}	.001^{NS}	1.004^{NS}
SR * CR	22.759*	.099^{NS}	3.279^{NS}	11.886^{NS}	51.590*	.086^{NS}	.094^{NS}	13.504*
MPP * CR	15.226*	.061^{NS}	.038^{NS}	.952^{NS}	3.780^{NS}	4.803^{NS}	4.980^{NS}	.300^{NS}
RE * UD * SR	1.059^{NS}	.061^{NS}	.000^{NS}	1.511^{NS}	10.850^{NS}	4.346^{NS}	6.046^{NS}	9.446^{NS}
RE * UD * MPP	8.026^{NS}	5.191^{NS}	7.988^{NS}	.112^{NS}	2.724^{NS}	2.083^{NS}	.258^{NS}	2.083^{NS}
RE * UD * CR	5.722^{NS}	6.825^{NS}	.991^{NS}	5.283^{NS}	4.153^{NS}	3.859^{NS}	.064^{NS}	.046^{NS}
RE * SR * MPP	.026^{NS}	.698^{NS}	.438^{NS}	4.180^{NS}	.632^{NS}	1.969^{NS}	1.230^{NS}	.502^{NS}
RE * SR * CR	10.544^{NS}	6.755^{NS}	16.775*	5.786^{NS}	8.370^{NS}	.731^{NS}	8.486^{NS}	.112^{NS}
RE * MPP * CR	.161^{NS}	2.220^{NS}	.005^{NS}	.004^{NS}	.455^{NS}	.315^{NS}	1.086^{NS}	1.352^{NS}
UD * SR * MPP	.446^{NS}	1.215^{NS}	9.904^{NS}	22.631*	11.385^{NS}	11.979^{NS}	5.283^{NS}	6.379^{NS}
UD * SR * CR	11.072^{NS}	12.683^{NS}	5.438^{NS}	4.744^{NS}	1.336^{NS}	34.258*	13.31^{NS}	18.862*
UD * MPP * CR	10.458^{NS}	.000^{NS}	3.088^{NS}	.731^{NS}	.455^{NS}	20.886^{NS}	11.07^{NS}	1.414^{NS}
SR * MPP * CR	14.811*	.107^{NS}	2.593^{NS}	3.500^{NS}	2.064^{NS}	.362^{NS}	.446^{NS}	.058^{NS}
RE * UD * SR * MPP	3.401^{NS}	3.041^{NS}	12.211*	.483^{NS}	.632^{NS}	2.972^{NS}	.464^{NS}	6.652^{NS}
RE * UD * SR * CR	12.922*	.083^{NS}	.888^{NS}	1.786^{NS}	4.208^{NS}	1.446^{NS}	.601^{NS}	17.494*
RE * UD * MPP * CR	1.715^{NS}	1.733^{NS}	1.528^{NS}	.258^{NS}	4.263^{NS}	.952^{NS}	.130^{NS}	7.286^{NS}
RE * SR * MPP * CR	1.086^{NS}	7.323^{NS}	4.263^{NS}	4.980^{NS}	1.018^{NS}	.206^{NS}	1.931^{NS}	1.894^{NS}
UD * SR * MPP * CR	5.283^{NS}	1.045^{NS}	1.275^{NS}	1.612^{NS}	.010^{NS}	3.652^{NS}	3.500^{NS}	8.178^{NS}
RE * UD * SR * MPP * CR	1.143^{NS}	.189^{NS}	.005^{NS}	5.344^{NS}	19.861*	.900^{NS}	2.529^{NS}	18.746*

FMID: Functional Multi-grain *Idli*